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Review Article

MICROBUBBLES - A POTENTIAL ULTRASOUND TOOL IN DRUG DELIVERY

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ABSTRACT

Microbubbles have been recently introduced as a promising drug delivery platform for ultrasound guided drug delivery. Ultrasound, traditionally used in diagnostic medicine, is finding a place in drug delivery in connection with these microbubbles. In addition to their non-invasive nature and the fact that they can be focused on targeted tissues, acoustic waves have been credited with releasing pharmacological agents from microbubbles, as well as rendering cell membranes more permeable. The microbubbles have an average size less than that of red blood cells, so they are capable of penetrating even into the small blood capillaries and releasing drug and genes under the action of ultrasound field after reaching the specific area of interest.This drug delivery and subsequent rupturing of microbubbles using localized ultrasound energy results in high local concentration of drug. Recently, targeting ligands are attached to the surface of the microbubbles, which have been widely used in cardiovascular system and tumor diagnosis and therapy. This review focuses on the characteristics of the microbubbles that give diagnostic and therapeutic properties, some important aspects of ultrasound parameters that are known to influence microbubble-mediated drug delivery.

Keywords: Microbubbles, Ultrasound, Ultrasound contrast agent, Drug delivery.

INTRODUCTION

The general goal of drug delivery and targeting is to improve the efficacy of drug action in the region of the disease while reducing undesired side effects, such as toxicity, in the healthy tissues. Lately, studies that combine drugs with an externally applied "trigger" are gaining attention. This approach controls drug action and/or deposition in the targeted region by an external energy field, such as light (photodynamic therapy), neutron beam (boron neutron capture therapy), magnetic field (targeted accumulation of magnetic drug carrier in the tissues close to the magnet), or mechanical energy. In order to improve the delivery of drugs and therapeutic genes, mechanical energy has been applied in the form of ultrasound irradiation.

Microbubbles are miniature gas bubbles of less than 50 micron diameter in water. The microbubbles which mostly contain oxygen or air can remain suspended in water for extended period. Gradually, the gas within microbubbles dissolved into water & bubbles disappear due to their compressibility, they undergo volumetric oscillation & scatter. Much more energies than rigid spheres of same size would do[1]. Coated microbubbles have advantage of being stable in body for a significant period of time; as shells serves to protect the gases of microbubbles from diffusion into blood stream. Microbubbles are sensitive to destruction by ultrasound. In process of destruction; the gas to liquid interface may achieved linear speed of about 700 m/s[2]. In such condition, closely located liposomes may be ruptured; and their contain release and/or deposited in surrounding cells & tissue[3]. Additional advantage could be gained by modulation of nearby cell permeability after microbubble destruction so the uptake of delivered drug would increase[4].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[5]. Two factors which are taken into account for drug delivery are:

1. Incorporation of drug into these microbubbles
2. Drug release from these microbubbles: Drug molecules can be incorporated in a variety of ways within the microbubble as follows[6, 7, 8]

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

Fig 1: Release Of Agents From Regular Bubbles And Microbubbles

COMPONENTS OF MICROBUBBLES [9, 10, 11, 12, 13]

Microbubbles basically comprise of three phases
1) Innermost Gas Phase
2) Shell Material Enclosing the Gas Phase
3) Outermost Liquid or Aqueous Phase

1) GAS PHASE

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

examples of second gas are per fluorocarbons or sulfur hexafluoride.

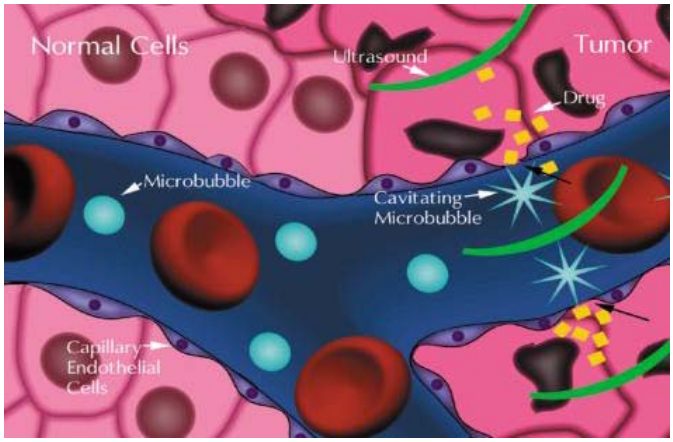

Fig 2: Drug Release From Microbubbles By Cavitation

2) SHELL MATERIAL

The shell material encapsulates the gas phase. It plays a major role in the mechanical properties of microbubble as well as diffusion of the gas out of the microbubble. The shell also acts a region for encapsulation of drug molecules also ligands can be attached to the shell membrane so as to achieve targeting of these microbubbles to the various 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.

Eg: The various types of shell materials that can be used are: proteins like albumin, carbohydrates like galactose, phosholipids like phosphotidylcholine, etc.

3) AQUEOUS OR LIQUID PHASE:

The external, continuous liquid phase in which the bubble resides typically includes a surfactant or foaming agent. Surfactants suitable for use include any compound or composition that aids in the formation & maintenance of the bubble membrane by forming a layer at the interphase. The foaming agent or surfactant may comprise a single component or any combination of compounds, such as in the case of co surfactants. Also the persistence of microbubble in body is inversely proportional to La Place pressure which in turn is directly proportional to surface tension of bubble. In other words decrease in the surface tension acting on the bubble increases the persistence time of the bubble in the body.

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

- Nonionic Surfactants: Polyoxyehylene polyoxypropylene copolymers Eg. Pluronic F-68, polyoxyethylene stearates, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerolpolyethylene glycol oxystearates, glycerol polyethylene glycol ricinoleate etc.
- Anionic Surfactants: Fatty acids having 12 -24 carbon atoms Eg. Sodium Oleate.

4) OTHER COMPONENTS:

The various other components that may be incorporated in the formulation include osmotic agents, stabilizers, chelators, buffers, viscosity modulators, air solubility modifiers, salts & sugars can be added to fine tune the microbubble suspensions for maximum shelf life & contrast enhancement effectiveness. Such considerations as sterility, isotonicity & biocompatibility may govern the use of such conventional additives to injectable compositions. Fig: 3 and Fig:4 explains the components of microbubbles.

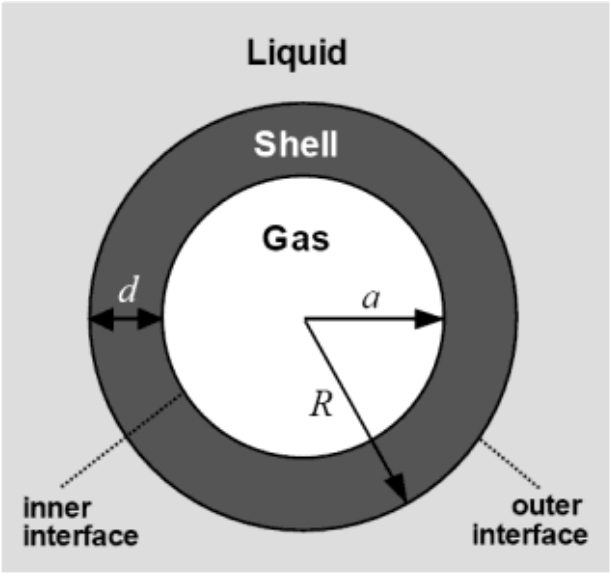

Fig 3: components of microbubbles

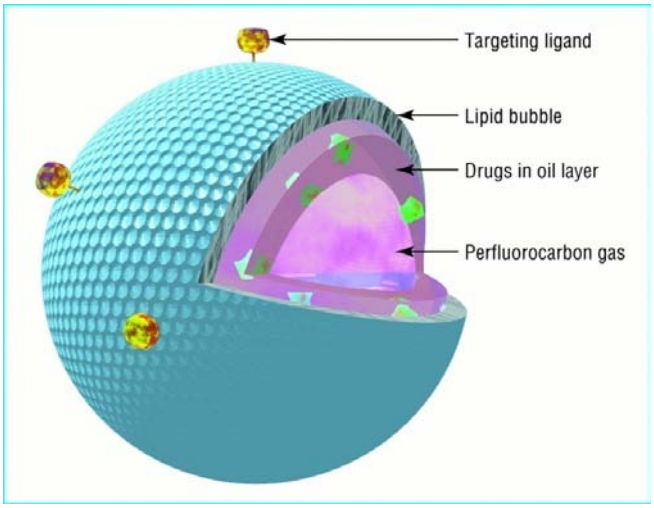

Fig 4: Various Layers In Microbubble Composition

PROPERTIES OF MICROBUBBLES

The ideal properties of microbubbles of divided into 2 classes.

1. FUNCTIONAL PROPERTIES [9, 14,15,16,17]

The functional properties are those which can render them useful for performing their various functions; Such as:-

a)Injectability: Since microbubbles are to be injected in the body to exert there effects they must be injectable.

b)Ultrasound scattering effeciency: As microbubbles become larger they become more ecogenic(the ultrasound sactteriing cross section of microbubbles is directly proportional to 6th powder of its radius at low ultrasound intencity,The stiff polymetric shell will not oscillate actively as ultrasound pressure increased above threshold value shell defects or cracks will form through which encapsulated gas can escape.

c) Biocompatibility: Microbubbles intract with vital organ of body at cellular levels they should be biocompatables.

d) Rheology: Microbubble possess rheologic property similar to red blood cells, Bubble perfusion data reflect red blood cell flow.

2. STRUCTURAL PROPERTIES[18, 19]

This refers to the structure or physical properties of microbubbles as follows

a) It should have an average external diameter between the ranges of 1 to 10 micrometer narrow size distribution so as to avoid complication when injected into the body.

b) Density & compression difference between themselves and the surrounding body tissues to create an acoustic impendance an to scatter ultrasound at a much higher intensity than the body tissue so as to be used as contrast agent.

c) The microbubbles acoustic back scatter signal is dependent on the compressibity of the gas,the size of microbubbles the thickness,viscocity and density of the bubble shell.The properties f surrounding medium and the frequency & power of the applied ultrasound

d) At even higher acoustic pressure the microbubbles undergoes forced expansion and compression,which result in its destruction by either outword diffusion of the gas during the compression Phase or diffusion via large shell defects,or by complete.Fragmentation of the microbubble shell and the gas core.

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

f) Uniformity of the shell thickness.The shell material also affects microbubble mechanical elasticity.The more elastic material,the more acoustic energy it can withstand before bursting.

METHODS TO PREPARE MICROBUBBLES[20, 21, 22, 23]

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.

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. It is the droplet size that will determine the size of the microbubble. As solvents volatilizes, polymer conc. in the droplet increases to a point where it precipitates in the presence of the less volatile nonsolvent. This process forms a film of polymer at the surface of the emulsion droplet. As the process continues, an outer shell wall is formed which encapsulates an inner core of nonsolvent liquid. Once complete, the resulting microcapsules can then be retrieved, washed & formulated in a buffer system. Subsequent drying , preferably by freeze-drying, removes both the nonsolvent organic liquid core and the water to yield air filled hollow microbubbles.

3) ATOMIZATION AND RECONSTITUTION

A spray dried surfactant solution is formulated by atomizing a surfactant solution into a heated gas this result 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 transmitting septum or by penetrating a septum with ultrasound probe including ultrasonically vibrating hypodermic needle. sonication can be accomplished in a number of ways for example: 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 patient.

Sonication can also be done within a syringe with a low power ultrasonically vibrated aspirating assembly on the syringe.

CHARACTERISATION OF MICROBUBBLES[21]

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

1)Microbubble Diameter & Size Distribution: The average diameter as well as size distribution of these microbubbles can be determined by Laser light Scattering, Scanning Electron Microscopy, Transmission Electron Microscopy.

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

3) Microbubble Concentration: The microbubble concentration is determined by counting the no. of microbubbles per ml by using the Coulter Counter Machine.

4) Air Content by densitometry: The content of air encapsulated within the microbubbles in the suspension samples is measured by oscillation U-tube densitometry.

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

MECHANISMS FOR TARGET DRUG DELIVERY USING MICROBUBBLES

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[24]. In a similar way, microbubbles can directly take up genetic material, such as plasmids and adenovirus[24,25], and phospholipid-coated microbubbles have a high affinity for chemotherapeutic drugs[26]. 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[27, 28].

The mechanisms by which ultrasound facilitates the delivery of drugs and genes result from a complex interplay among the therapeutic agent, the microbubble characteristics, the target tissue , and the nature of ultrasound energy. 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[29,30]. It has been reported that the application of ultrasound to contrast agents creates extravasation points in skeletal muscle capillaries[31,32], 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[33] demonstrated in an exteriorized spinotrapezius preparation that ultrasonic destruction of gas-filled microbubbles caused rupture of microvessels with diameter ≤ 7 μm (capillaries), with local extravasation of red blood cells. To broaden the spectrum of drugs associated with bubbles to include proteins, enzymes, antibodies and hydrophilic substances, wants to improve loading capacity, or if the drug can't survive the harsh conditions of microbubble preparation, one could use multi-particle assemblies. Liposomes or nanoparticles that entrap the drug can be prepared separately and then coupled to the surface of the microbubbles[34,35,36,37]Both lipid- and polymer shelled microbubbles could be used. When the microbubble is destroyed by ultrasound in the target tissue, the energy released in the form of high shear flow, microjets, and microstreaming, will cause rupture of the membrane of microbubble-associated liposomes and subsequently release the encapsulated drug[38] . FIG: 5 explain the drug delivery mechanism from microbubbles.

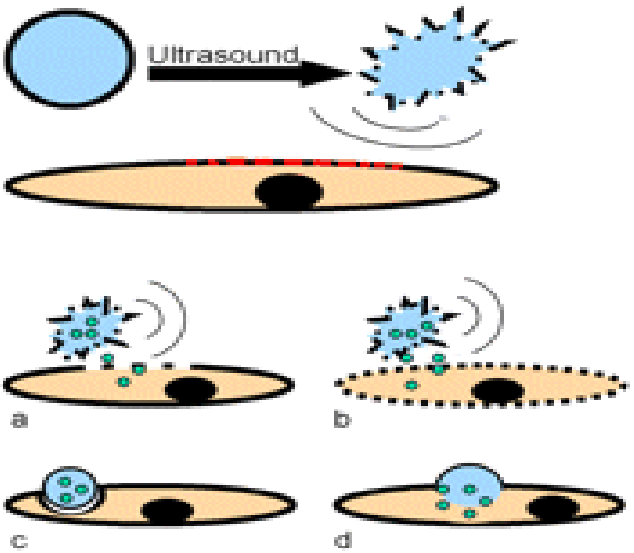

Fig 5: Destruction of Microbubbles by Ultrasound Resulting In Increased Membrane Permeability

Destruction of microbubbles by ultrasound resulting in increased membrane permeability by shear stress, temperature rise and activation of reactive oxygen species, Drug delivery from microbubbles is by a) transient holed induced by shear stress b) increase in membrane fluidity c) endocytosis of microbubbles d) fusion of the microbubble membrane with the cell membrane

APPLICATIONS

- In gene therapy
- In liver imagine :
- In cardiac diseases

OTHER APPLICATIONS

1) Biochemical application: [39, 40-44]

a) Diagnostic Aids: microbubbles are elastic and compressible, these undergo compression and rarefaction thereby creating an acoustic impedance mismatch between biological tissues and fluids as these are efficient reflectors of ultrasound, hence used as contrast agents.

These are used as diagnostic aids for:

- Organ Edge Delineation
- Blood Volume and Perfusion
- Inflammation
- Cancer
- Liver
- Also used to scan the tumors arising in the body.
- Used for imaging the gall bladder stone .

2) In inflammation: Inflammation and immune reaction are caused and sustained by inflammatory cells and migrates from the circulation through the endothelial layer to the target tissue. Adhesion molecules have become interesting targets to study inflammation processes. Several antibodies have developed to target ICAM-1, VCAM-1, P- and L-selectin and others[45-47]

3) Plaque: Targeted microbubbles, ICAM-1 and VCAM-1 could be successfully localized in-vivo and in murine models of atherosclerotic disease[48-50] this may lead to future application in atherosclerotic plaque.

4) Thrombus: Its application is increased in diagnosis of deep vein or intracardiac thrombi. Labella targeted microbubbles have been shown to attach to clots *in-vitro* and *in-vivo*[51]

5) General applications:

i. 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.

ii. Micro bubbles can penetrate deeply into skin for a good scrub without the need for any shampoo or soap. The baths with microbubbles are especially helpful for pets which have skin allergies to pet shampoos.

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