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System for the Microbubble-Mediated and Acoustically Targeted Delivery of Drugs

Krishna Chandra Balodi[1], Himanshu Rathore[2]

Assistant Professor[1], Student[2]

Department of Pharmaceutical Science

Rofel Shri G M Bilakhia College of Pharmacy

Corresponding Author's Email id: *himanshurrathore5@gmail.com[2]*

Abstract

Acoustic targeted drug delivery delivers pharmaceuticals to a specific location in the body using high intensity focused ultrasound waves. It is a revolutionary approach that uses ultrasonic waves with frequencies exceeding human hearing or over 20,000 Hz to assist medicine delivery to a specific spot in the body. Microbubbles, on the other hand, are cavitating gas entities that act as mediators for the concentration of ultrasonic energy and generate forces that increase the permeability of cell membranes and break drug carrier vesicles. Combining acoustic medication delivery with microbubbles improves drug distribution to targeted tissues and increases activity owing to cell membrane permeabilization. These microbubbles can carry a variety of medications, genetic material, proteins, and tiny chemical agents. The purpose of this paper is to discuss the design and advantages of microbubble-mediated acoustic targeted medication delivery.

Keywords: *Microbubble, Cavitation, Gene Delivery, Microbubbles, Ultrasound, Targeted Drug Delivery.*

INTRODUCTION

The acoustic targeted medication delivery system delivers pharmaceuticals to any specific place in the body using high intensity focused ultrasound radiation. It is a revolutionary approach that employs ultrasonic waves with frequencies higher than human hearing, i.e. above 20,000 Hz, to enhance medicine delivery to a specific region in the body. Such frequencies allow

ultrasound to penetrate the body without considerable signal attenuation or distortion.

Good quality body images along with good spatial resolution can be formed for diagnostic purposes. Low energy ultrasound (0.1-100mW/cm²) is used for diagnostic imaging while higher energy ultrasound (100-10,000W/cm²) is used for non-invasive therapies.

Ultrasound therapy is the delivery of acoustic energy in a small (1 to 10 millimetres) focal region, resulting in a variety of therapeutic effects such as thermal tissue coagulation, kidney stone comminution (lithotripsy), mechanical tissue disruption (histotripsy), bone healing, neural activity modulation, and many others. Their concentrating power enables therapy in a confined zone, resulting in less injury to adjacent healthy tissues. Furthermore, the non-invasive method considerably minimises the possibility of problems.

Properties of Ultrasound Waves

1. Ultrasound waves are physical in nature.
2. They can be reflected, refracted, focused and absorbed.
3. They arise due to actual movements of molecules due to compression and expansion of the medium.
4. They have an ability to physically act upon biomolecules and cells.

MICROBUBBLES

Microbubbles are microspheres floating in a liquid carrier phase that are filled with air or gas. Surfactants in the liquid phase regulate the surface characteristics as well as the stability of the bubbles. These act as cavitating gas bodies, acting as mediators for the concentration of ultrasonic energy and producing the forces necessary for increasing cell membrane permeability and destroying carrier vesicles. Microbubbles do not agglomerate and stay unique from one another. Their sizes range from 1 to 100 m. In general, such microbubbles contain oxygen or air and may float in water for a lengthy period of time. Over time, the oxygen/gas dissolves into water, and the bubbles vanish.

Microbubble Properties Microbubbles have functional and structural features that are addressed in depth below.

Functional Properties

Functional properties are those that make them beneficial in carrying out their functions. They are as follows:

1. **Injectability:** In order to exert their various actions, they must be readily injectable into the body.
2. **Ultrasound Scattering Efficiency:** They must have ability to scatter ultrasound as they act in combination with ultrasound waves.
3. **Biocompatibility:** Microbubbles interact with the vital organs of the body at cellular levels. Hence they should be biocompatible and safe.

Structural Properties

These properties refer to the physical properties and structure of the microbubbles. They include:

1. Average external diameter: It should range between 1-10 µm with narrow size distribution so as to avoid complications when injected.
2. Density and compressibility: There should be density and compressibility difference between microbubbles and surrounding body tissues so as to create acoustic impedance and to scatter ultrasound at much higher intensities than body tissues.
3. Ligand affinity: Microbubbles should be capable of being modified for attachment of various ligands to target them to specific tissues or organs.
4. Shell thickness: Microbubble thickness should be uniform for maximum stability.

CONSTITUTION OF MICROBUBBLE SYSTEM

Microbubble system comprises of three phases:

1. Innermost gas phase
2. Shell material enclosing the gas phase
3. Outermost aqueous phase

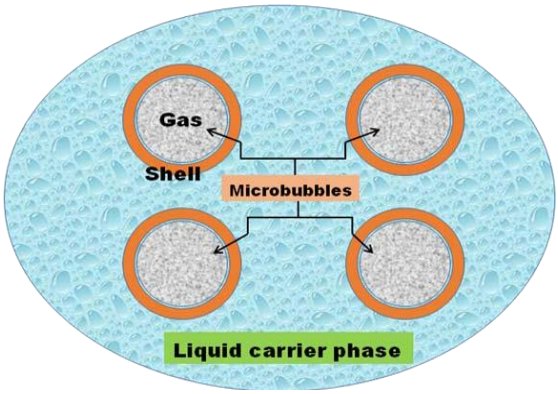

Figure:-1

In addition to this, the system may also comprise of various other components which are listed further.

1) Gas Phase

It is the innermost phase and may include a single gas or combination of gases. The advantage of combination of gases is that they cause differentials in partial pressure and generate gas osmotic pressures which stabilize the bubbles. In combination, two types of gases are involved. One is the primary modifier gas which is also known as first gas. Air is mainly used as first gas, sometimes nitrogen is also used as first gas. The vapour pressure of first gas is (760-x) mm of Hg, where x is the vapour pressure of second gas. The other gas is gas osmotic agent and is less permeable through bubble surface than the first gas. Selection of second gas should be such that it is less soluble in blood and serum. It should have sufficient partial vapour pressure at the temperature of use to provide the desired osmotic effect. Commonly used gases include perfluorocarbons or sulphur hexafluoride.

2) Shell Material

It encapsulates the gas phase and plays a major role in imparting mechanical properties to the microbubble. It aids in diffusion of gas out of microbubble. It also acts as region for encapsulation of drug molecules. Various ligands can be attached to shell membrane so as to achieve targeting of these microbubbles to various organs or tissues. The shell material also accounts for elasticity and compressibility of microbubbles. When the shell material is highly elastic, the microbubble is capable of withstanding large acoustic energy before bursting. This increases the residence time of bubbles in the body. Hydrophilic shell material leads to rapid dissolution of the microbubbles thereby decreasing the residence time of bubbles in the body.

Various types of shell materials can be used and include

- Proteins like albumin
- Carbohydrates like galactose
- Phospholipids like phosphotidylcholine, phosphotidylethanolamine, etc.
- Biodegradable polymers like PVA, polycaprolactone, etc.

3) Outermost Aqueous Phase

It is the external continuous liquid phase in which the microbubbles reside. Surfactant or foaming agent is present in the aqueous phase. Surfactants aid in formation and maintenance of bubble membrane by forming a layer at interface. These

decrease the surface tension acting on bubble thereby increasing the persistence time of the bubble in the body. The foaming agent or surfactant may comprise a single component or combination of surfactant with co- surfactants. These include

- Block copolymers of polyoxypropylene, polyoxyethylene, sugar esters, fatty alcohols, aliphatic amine oxides, hyaluronic acid esters and their salts, etc.

- Nonionic surfactants such as Pluronic F-68, polyoxyethylene stearates, polyoxyethylene fatty alcohols ethers, glycerol polyethylene glycol oxystearates, etc.

- Anionic surfactants: Sodium oleate.

4) Other Components

Osmotic agents, stabilizers, chelating agents, buffers, viscosity modulators, air solubility modifiers, salts and sugars can be added to fine tune the microbubble suspensions for maximum shelf life and contrast enhancement effectiveness. Sterility, isotonicity, biocompatibility limit the use of several conventional additives to these injectable preparations.

CHARACTERIZATION OF MICROBUBBLES

Evaluation parameters for the characterization of microbubbles are as follows:

1. Microbubble diameter and size distribution: Techniques such as laser light scattering, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) can be used to determine average diameter and size distribution of microbubbles.
2. Shell Thickness: Determination of shell thickness can be done by coating the microbubble shell with a fluorescent dye such as Red Nile, which is then determined by fluorescent microscopy against dark background.
3. Microbubble concentration: The microbubble concentration is determined by counting the number of microbubbles per ml by using Coulter Counter.
4. Air content by densitometry: Air content encapsulated within the microbubbles is measured by oscillation U-tube densitometry with a digital density meter. The instrument is calibrated with air and purified water prior to use. Density of the medium 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:

$$C_{air} = \frac{\rho_1 - \rho_2}{\rho_2} X\, 100$$

Where,

Cair is air content (%v/v); ρ1 (g/ml) is density before elimination of encapsulated air; ρ2 (g/ml) is density after elimination of encapsulated air.

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

CAVITATION PHENOMENON

Cavitation is the formation and/or activity of gas filled bubbles in a medium exposed to ultrasound. When a liquid is treated with high-intensity ultrasonic waves, the sound waves propagate into the liquid and produce alternating high-pressure (compression) and low-pressure (expansion) cycles. This leads to expansion and contraction of gas bubbles due to pressure waves generated by ultrasound. If the oscillation in bubble size is fairly stable (repeatable over many cycles), it is known as stable/ non-inertial cavitation (Figure 2(a)). Such oscillation creates a circulating flow of fluid (microstreaming) around the bubble [19-. As ultrasound intensity increases, the amplitude of oscillation increases. During the rarefaction part of the cycle, the pressure in the wave is below the ambient pressure, and gas pockets expand until the pockets collapse violently and implode due to the high stresses developed in the walls (Figure 2(b)). This phenomenon is known as transient/ inertial/ collapse cavitation. Such collapse cavitation is detrimental to cells or vesicles in its vicinity due to shock waves produced by collapse of bubbles and free radicals produced due to increased temperature. Collapsed bubble fragments into smaller bubbles that serve as cavitation nuclei, grow in size and again collapse. If collapse is near a solid surface, it can cause ejection of a liquid jet at sonic speed towards the surface. In proximity to blood vessel wall, skin and large cell, the liquid jet pierces the surface thereby damaging the tissues. Increased intensity and decreased frequency of ultrasound leads to increased intensity of collapse cavitation. Bubble size, gas species, interfacial tension, and surface rigidity also affect cavitation process.

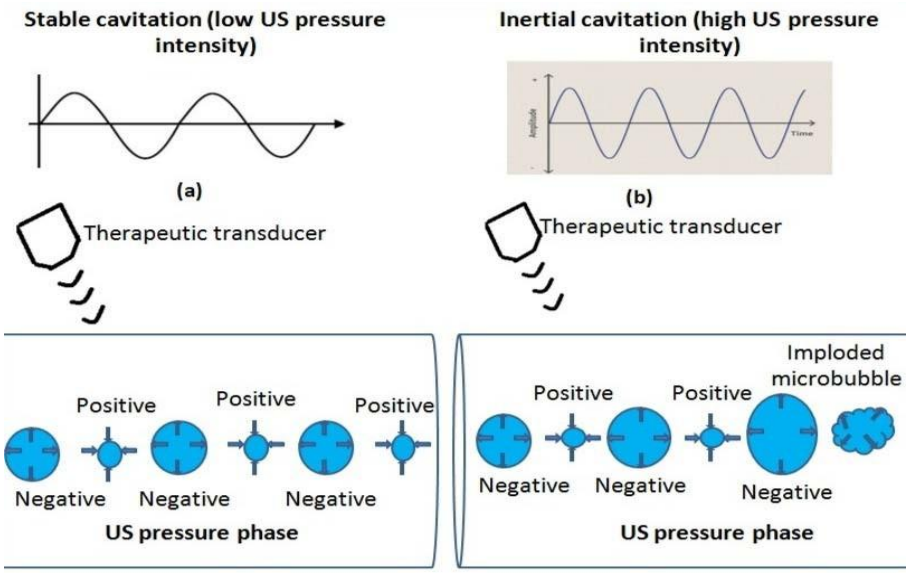

Fig.2: Types of cavitation (a) Stable cavitation (b) Inertial cavitation

INNOVATIONS IN DRUG DELIVERY

Ultrasound Induced Drug Delivery

Enhanced Transport: Exposure of fluid to ultrasound i.e. insonation of fluid leads to generation of oscillatory motion in the fluid due to ultrasound pressure waves and is responsible for this phenomenon. Oscillating fluid increases the effective diffusivity of molecules. Thus, transport of any drug, free or bound to carrier will be augmented by oscillatory motion of nearby fluid. Such ultrasound enhanced transport may occur within blood, cells or extracellular fluids [1].

Perturbation of Drug Carrier: Disruption of drug carriers can be induced by ultrasound. Vesicles more dense than the surrounding liquid will be sucked into the shear field surrounding an oscillating bubble. If shear stress exceeds the strength of vesicle, the vesicle will rupture and spill its contents releasing the drug at the target site [1].

Cell Permeabilization and Capillary Rupture: Cells in the environment of cavitation events are subject to shear from microstreaming, shock waves and sonic jets. Collapse of microbubble near a capillary or blood vessel wall will cause the liquid jet to shoot right into the wall leading to rupture of cell membrane leading to increased permeability [22-25].

Miscellaneous: Diagnosis of various diseases is commonly done by ultrasound technology due to its beneficial characteristics such as non- invasiveness, small device size, simple and real time

operations and low costs . Calculi, tumors, bone fractures,

Parkinson disease are some of the conditions where ultrasound technology can be used therapeutically. Ultrasound devices have been applied in newer areas in recent years (Figure 3). Also, catheter-based ultrasound and MRI-guided focused ultrasound are being utilized in clinical settings.

Computer-controlled ultrasound systems allow precise exposure to the target sites. Such devices are used in the thrombolytic treatment of cerebral infarct, pulmonary embolism, deep venous thrombosis, hepatic cancer, prostate cancer, breast cancer and uterine fibroids [26]. Further, combination of ultrasound and microbubbles has reportedly enhanced gemcitabine treatment of inoperable pancreatic cancer in clinical trials.

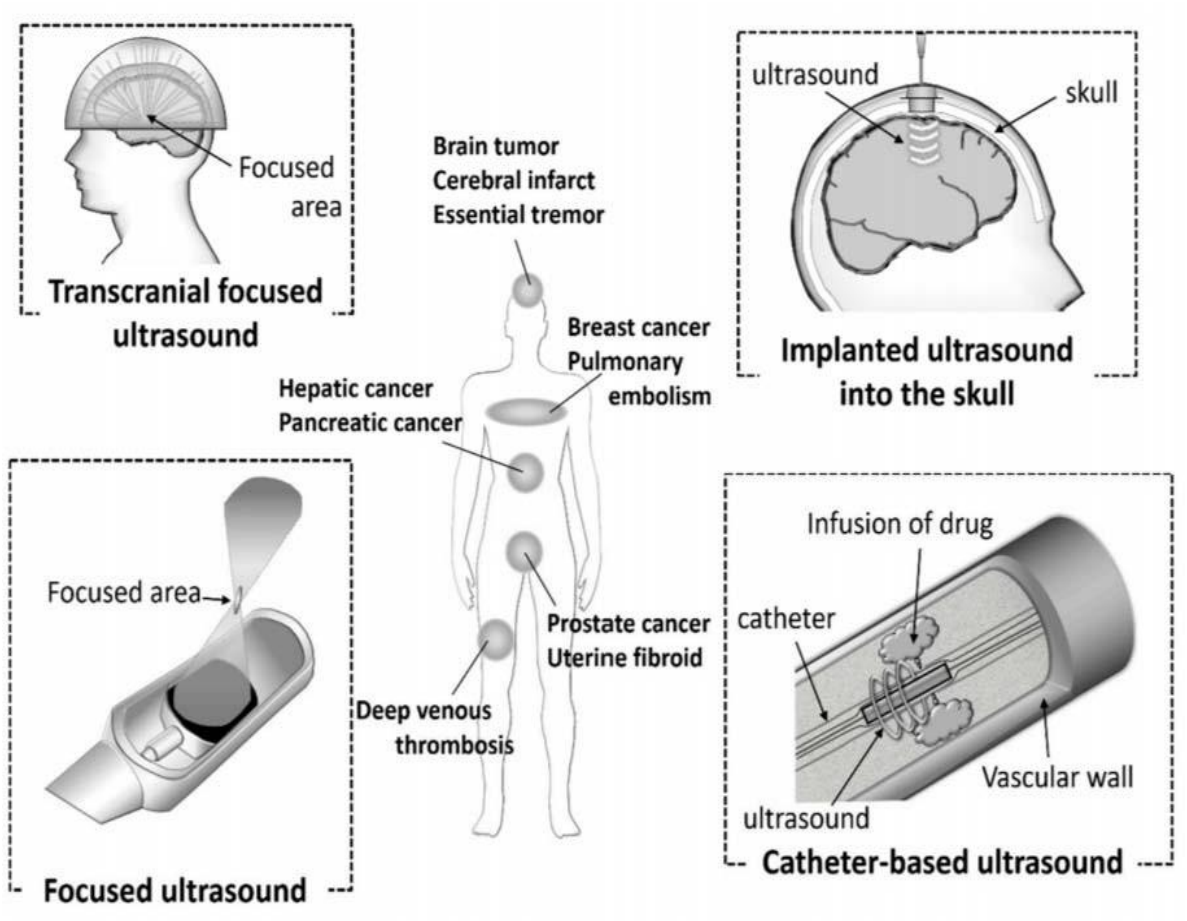

Fig. 3: Ultrasound devices used in clinical settings (reproduced from: Endo-Takahashi & Negishi, Pharmaceutics, 2020;12:964)

A transcranial focused ultrasound system for the treatment of crucial cancers has been authorised. Sonodynamic treatment is also performed using ultrasound instruments (SDT). SDT is a cancer treatment in which the sensitizer stored in tumour cells is activated by ultrasound, resulting in the production of free radicals. In comparison to photodynamic treatment, SDT can enable deeper penetration into cancer cells (PDT).

Depending on the location of the tumour, a combination of sonodynamic and photodynamic treatment (SPDT) may be used. In a clinical context, MRI-guided targeted low intensity ultrasound was utilised to administer chemotherapy drugs to a patient with a malignant brain tumour. A pulsed ultrasound device implanted into the patient's skull was used to deliver the chemotherapeutic drug to the glioblastoma.

Microbubble Mediated Acoustic Targeted Drug Delivery

1. Compression and rarefaction of microbubbles cause an acoustic impedance mismatch between biological tissues and fluid. As a result, they are utilised as contrast agents. They may also be utilised as diagnostic tools for organ delineation, blood volume and perfusion determination, inflammation, cancer, liver, tumours, and gall bladder stone imaging.
2. Cavitation occurs when microbubbles oscillate. The use of low frequency ultrasound causes bubbles to break, allowing medicine to be released from microbubbles and facilitating drug administration. Drugs can be given to specific areas in illnesses such as- Parkinson disease, Alzheimer's disease, brain tumor metastasis, etc. through the disruption of blood brain barrier.
3. Stem cell transplantation.
4. Inflammatory disorders
5. Thrombolytic drugs such as tissue plasminogen activator (tPA), urokinase can be targeted through microbubbles at thrombus anywhere in the body.
6. Cardiovascular diseases such as myocardial infarction can be treated by microbubble targeted drug delivery to the heart.
7. Gene delivery for treatment of cancer, cystic fibrosis, heart disease, diabetes and AIDS can be done by incorporating gene into microbubble.

CONCLUSION

Microbubbles have swiftly progressed from a diagnostic adjuvant to a potential therapeutic drug delivery method in recent years. They've showed a lot of promise in the treatment of inflammatory and

cancerous disorders. Furthermore, low power ultrasound technology is widely recognised for being non-invasive and capable of being utilised repeatedly for diagnostic and therapeutic applications. This review demonstrates the effectiveness of using microbubbles in combination with ultrasound to transport therapeutic chemicals and genes to target tissues. This review has gone through the combinatorial synergistic carrier system in great depth. Although no adverse effects have been observed, there have been questions raised about their safety.

Efforts are being made to further develop and test these carrier systems as a precondition to their effective adoption in clinical settings.

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