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Microbubble Mediated Acoustic Targeted Drug Delivery System

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ABSTRACT

Acoustic targeted drug delivery uses high intensity focused ultrasound energy for delivering drugs to a targeted site in the body. It is a novel technique that utilizes the ultrasonic waves having frequencies above human hearing or above 20,000 Hz to facilitate the release of drugs to the targeted location in the body. On the other hand, microbubbles are cavitating gas bodies, which serve as mediators for the concentration of ultrasound energy and produce forces that are responsible for increasing the permeability of cell membranes and disrupting the drug carrier vesicles. Combining acoustic drug delivery with microbubbles facilitates delivery of drugs to targeted tissues and facilitates enhanced activity due to permeabilization of the cell membrane. Several drugs, genetic material, proteins and various smaller chemical agents can be delivered by such microbubbles. This review focuses on the design and benefits of microbubble mediated acoustic targeted drug delivery.

Keywords*: Acoustic targeted drug delivery, Cavitation, Gene Delivery, Microbubbles, Ultrasound.*

INTRODUCTION

Acoustic targeted drug delivery system uses high intensity focused ultrasound energy for delivering drugs to any targeted site in the body [1]. It is a novel technique which uses ultrasound waves having the frequencies above human hearing i.e. above 20,000 Hz to facilitate drug release to the targeted location in the body. Such frequencies aid in penetration of ultrasound into the body without significant attenuation or distortion of the signal [2].

Good quality body images along with good spatial resolution can be formed for diagnostic purposes. Low energy ultrasound (0.1-100mW/cm^2) is used for diagnostic imaging while higher energy ultrasound (100-10,000W/cm^2) is used for non-invasive therapies [3].

Ultrasound therapy involves disposition of acoustic energy in a small (1 to 10 millimeters) focal region which causes various effects such as thermal tissue coagulation [4], kidney stone comminution (lithotripsy) [5], mechanical tissue disruption (histotripsy) [6], bone healing [7], modulation of neural activities [8] and many other therapeutic effects [9-11].

Their focusing capability allows treatment in a localized region facilitating minimized damage to the surrounding healthy tissues. Also, the non-invasive procedure reduces possible complications significantly.

Properties of Ultrasound Waves [1]

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 consist of microspheres filled with air or gas and suspended in a liquid carrier phase. The liquid phase contains surfactants that control the surface properties as well as stability of the bubbles [12]. These function as cavitating gas bodies which serve as mediators for the concentration of ultrasound energy and produce the forces responsible for increasing the permeability of cell membranes and disrupting the carrier vesicles. Microbubbles remain distinct from each other and do not agglomerate. Their size ranges from 1-100 µm. Generally oxygen or air is enclosed in such microbubbles which can remain suspended in water for extended period of time. Over a period of time, oxygen/gas dissolves into water and bubbles disappear [12,13].

Properties of Microbubbles

Microbubbles possess functional and structural properties which are discussed below in detail [13].

Functional Properties

Functional properties are those properties which render them useful for performing their functions. They include:

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 [13-16] (Figure 1):

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

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

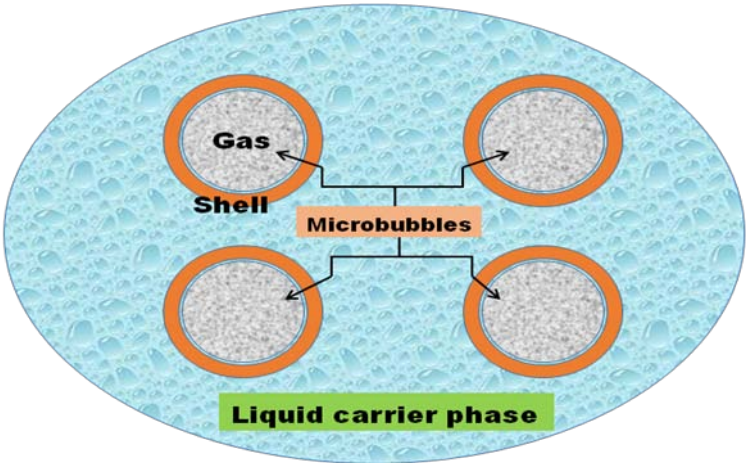

Fig. 1: Diagrammatic Representation of Microbubble System

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 [17]:

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,

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

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

ρ_2 (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 [18]. 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 [19-21]. 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.

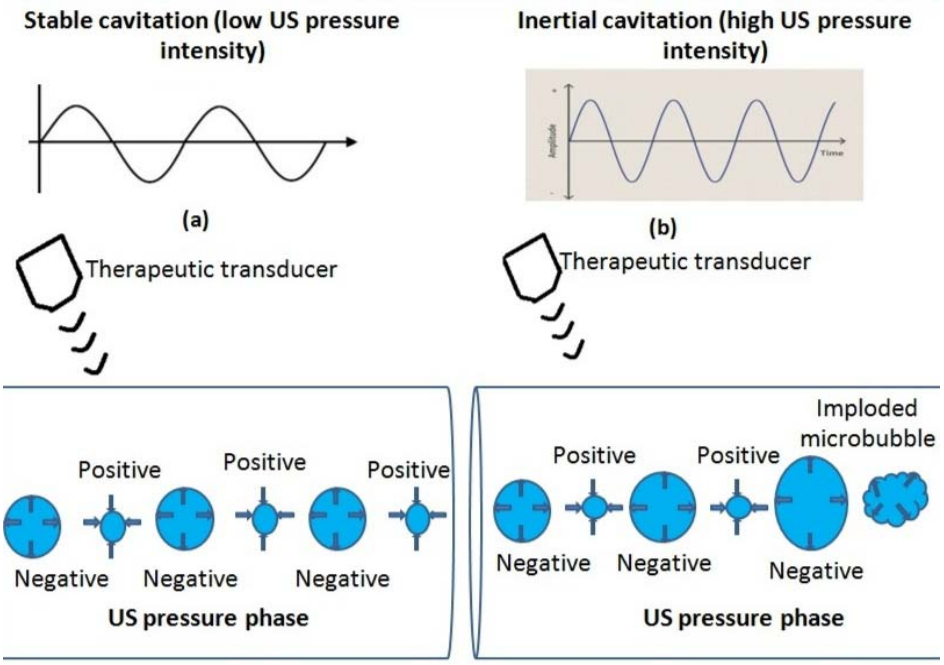

Fig.2: Types of cavitation (a) Stable cavitation (b) Inertial cavitation

INNOVATIONS IN DRUG DELIVERY

Ultrasound Induced Drug Delivery

1) **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].

2) **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].

3) **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].
4) **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 [26]. 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 [27].

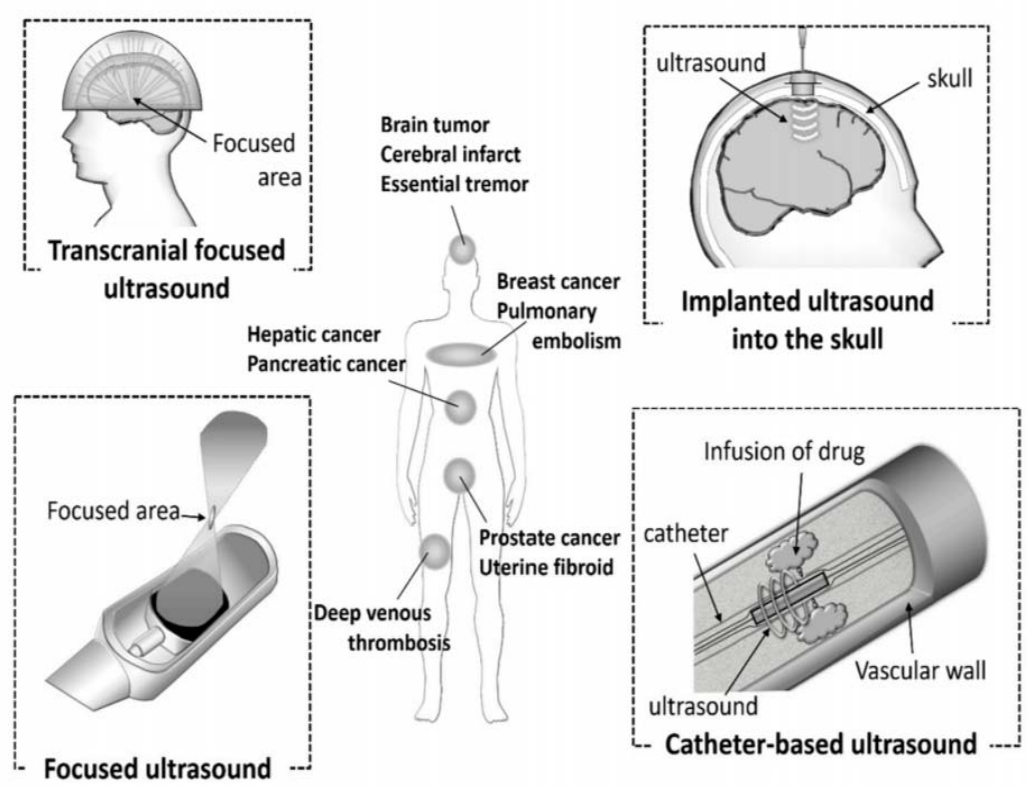

Fig. 3: Ultrasound devices used in clinical settings (reproduced from: Endo-Takahashi & Negishi, Pharmaceutics, 2020;12:964)

Transcranial focused ultrasound system has been approved for the treatment of essential tumors. Ultrasound devices are also used for sonodynamic therapy (SDT). SDT is a cancer therapy in which the sensitizer accumulated in the tumor cells is activated by ultrasound and produces free radicals. SDT can provide deep penetration to target cancer cells compared to photodynamic therapy (PDT). Combination of both sonodynamic and photodynamic therapy (SPDT) can be adopted depending on the area of tumor. In a clinical setting, the state-of-the-art MRI-guided focused low intensity ultrasound was used for a patient with a malignant brain tumor to deliver chemotherapeutic agents [28]. The chemotherapeutic agent was delivered to the glioblastoma using a pulsed ultrasound device implanted into the skull of the patient [29].

Microbubble Mediated Acoustic Targeted Drug Delivery

Microbubbles undergo compression and rarefaction creating an acoustic impedance mismatch between biological tissues and fluid. Due to this they are used as contrast agents. They can also be used as diagnostic aids for organ edge delineation, determination of blood volume and perfusion, inflammation, cancer, liver, tumors and gall bladder stone imaging [30-33].

Microbubbles oscillate and undergo cavitation. Application of low frequency ultrasound results in bursting of bubble leading to release of drug from microbubbles and facilitation of drug delivery. In this manner, drugs can be delivered to target sites in disorders such as-

1) Parkinson disease, Alzheimer's disease, brain tumor metastasis, etc. through the disruption of blood brain barrier.
2) Stem cell transplantation.
3) Inflammatory disorders
4) Thrombolytic drugs such as tissue plasminogen activator (tPA), urokinase can be targeted through microbubbles at thrombus anywhere in the body.
5) Cardiovascular diseases such as myocardial infarction can be treated by microbubble targeted drug delivery to the heart.
6) Gene delivery for treatment of cancer, cystic fibrosis, heart disease, diabetes and AIDS can be done by incorporating gene into microbubble.

CONCLUSION

In recent times, microbubbles have evolved rapidly from a diagnostic adjuvant to a possible therapeutic drug delivery system. They have shown great promise in the treatment of inflammatory and malignant diseases. Also, low power ultrasound technology is well known to be non-invasive and can be used repeatedly for diagnostic as well as therapeutic purposes.

This review points to the efficacy of microbubbles in conjunction with ultrasound in delivery of therapeutic agents as well as genes to target organs. The combinatorial synergistic carrier system has been explained in detail in this review. Although no incidence of harmful effects has been reported, concerns have been expressed as to their safety. Efforts are ongoing to develop and test further these carrier systems as a precursor to successful implementation in clinical settings.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

1) Pitt WG, Husseini GA, Staples BJ. *Ultrasonic Drug Delivery- A General Review*. Expert Opin Drug Deliv. 2004; 1(1): 37-56p.
2) Wang T, Wilson KE, Machtaler S, et al. *Ultrasound and Microbubble Guided Drug Delivery: Mechanistic Understanding and Clinical Implications. Curr Pharm Biotechnol*. 2014; 14(8): 743–52p.
3) Dubinsky TJ, Cuevas C, Dighe MK, et al. *High-intensity focused ultrasound:Current potential and oncologic applications.* AJR. 2008; 190(1): 191–9p.
4) Kennedy JE. *High-intensity focused ultrasound in the treatment of solid tumours.* Nat Rev Cancer. 2005; 5(4): 321–7p.
5) Rassweiler JJ, Knoll T, Kohrmann KU, et al. *Shock wave technology and application: an update.* Eur Urol. 2011; 59(5): 784–96p.
6) Roberts WW, Hall TL, Ives K, et al. *Pulsed cavitational ultrasound: a noninvasive technology for controlled tissue ablation (histotripsy) in the rabbit kidney.* J Urol. 2006; 175(2): 734–8p.
7) Erdogan O, Esen E. *Biological aspects and clinical importance of ultrasound therapy in bone healing.* JUM. 2009; 28(6): 765–76p.
8) Tyler WJ. *Noninvasive neuromodulation with ultrasound? A continuum mechanics hypothesis.* Neuroscientist. 2010; 17(1): 25–36p.
9) Miller DL. *Overview of experimental studies of biological effects of medical ultrasound caused by gas body activation and inertial cavitation.* Prog Biophys Mol. Biol. 2007; 93(1–3): 314–30p.
10) Nyborg WL. *Biological effects of ultrasound: development of safety guidelines. Part II: general review.* Ultrasound Med Biol. 2001; 27(3): 301–33p.
11) Baker KG, Robertson VJ, Duck FA. *A review of therapeutic ultrasound: biophysical effects.* Phys Ther. 2001; 81(7): 1351–8p.
12) Kurup N, Naik P. *Microbubbles: A Novel Drug Delivery System*. JPRHC. 2(3): 228-34p.
13) Patel R. *Microbubble: An ultrasound contrast agent in molecular imaging*. Pharma Times. 2008; 40:15p.
14) Maliwal D, Patidar V. *Microbubbles Contrast Agents Using Ultrasound*. RJPT. 2008; 1(3): 152-4p.
15) Eniola AO, Hammer DA. *Invitro characterization of leukocyte mimetic for targeting therapeutics to the endothelium using two receptors.* Biomaterials. 2005; 26: 7136-44p.
16) Eniola AO, Willcox PJ, Hammer DA. *Interplay between rolling and firm adhesion elucidated with a cell-free system engineered with two distinct receptor-ligand pairs*. Biophys J. 2003; 85: 2720-31p.
17) Bjerknes K, Sontaum PC. *Preparation of polymeric microbubbles: formulation studies and product characterisation*. IJP. 1997; 158: 129p.
18) Barnett SB, ter Haar GR, Ziskin MC, et al. *Current Status of Research on Biophysical Effects of Ultrasound.* Ultrasound Med Biol. 1994; 20(3): 205–18p.
19) Marmottant P, Hilgenfeldt S. *Controlled vesicle deformation and lysis by single oscillating bubbles.* Nature. 2003; 423(6936): 153–6p.
20) Elder SA. *Cavitation Microstreaming.* JASA. 1958; 31(1): 54–64p.
21) Nyborg WL. *Ultrasonic Microstreaming and Related Phenomena.* Br J Cancer. 1982 ;45(Suppl V): 156–60p.
22) Kruskal J, Goldberg S, Kane R. *Novel in Vivo Use of Conventional Ultrasound to Guide and Enhance Molecular Delivery and Uptake into Solid Tumors.* Annual Meeting of the Radiological Society of North

America. Chicago, IL RSNA; 2001 804p.
23) Song J, Chappell JC, QI M, et al. *Influence of injection site, microvascular pressure and ultrasound variables on microbubble-mediated delivery of microspheres to muscle.* JACC. 2002; 39(4): 726–31p.
24) Skyba DM, Price RJ, Linka AZ, et al. *Direct In Vivo Visualization of Intravascular Destruction of Microbubbles by Ultrasound and its Local Effects on Tissue.* Circulation. 1998; 98: 290–3p.
25) Schlachetzki F, Holscher T, Koch HJ, et al. *Observation on the integrity of the blood-brain barrier after microbubble destruction by diagnostic transcranial color-coded sonography.* J Ultrasound Med. 2002; 21(4): 419–29p.
26) Endo-Takahashi Y, Negishi Y. *Microbubbles and Nanobubbles with Ultrasound for Systemic Gene Delivery.* Pharmaceutics. 2020; 12: 964p.
27) Dimcevski G, Kotopoulis S, Bjånes T, et al. *A human clinical trial using ultrasound and microbubbles to enhance gemcitabine treatment of inoperable pancreatic cancer.* JCR. 2016; 243: 172-81p.
28) https://sunnybrook.ca/media/item.asp?c=1&i=1351&page=33939&f=blood-brain
29) Carpentier A, Canney M, Vignot A, et al. *Clinical trial of blood-brain barrier disruption by pulsed ultrasound.* Sci Transl Med. 2016; 8: 343p.
30) Klibanov AL. *Targetted delivery of gas filled microspheres, contrast agents for ultrasound imaging:* Adv Drug Deliv. Rev. 1999; 37: 139-57p.
31) Klibanov AL. *Ligand carrying gas filled microbubble : Ultrasound contrast agents for targeted molecular imaging.* Bioconjug Chem. 2005; 16: 9-17p.
32) Lindner JR. *Microbubbles in medical imaging : Current applications and future directions.* Nat Rev Drug Discov. 2004; 3: 527-32p.
33) McCulloch M, Gresser C, Moos S. *Ultrasound contrast physics : A series on contrast echocardiography, article 3.* JASE. 2000; 13(10): 959-67p.