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Review Article

Micro bubbles: a new era in ultrasound-a Review

Dr Parita CK[1], Dr Girish K[2], Dr Ambika P[3], Dr Sharashchandra MB[4], Dr Nikhat A[5], Dr Bhavani R[6]

[1]Professor, Department of Oral Medicine and Radiology, Albadar Rural Dental College and Hospital , Gulbarga
[2]Principal, Professor and Head of the Department, Department of Oral Medicine and Radiology, Albadar Rural Dental College and Hospital , Gulbarga
[3]Post Graduate Student, Department of Oral Medicine and Radiology, Albadar Rural Dental College and Hospital , Gulbarga
[4]Reader, Department of Periodontology and Oral Implantology, S B Patil Institute for Dental Sciences, Bidar
[5]Post Graduate Student, Department of Oral Medicine and Radiology, Albadar Rural Dental College and Hospital , Gulbarga
[6]Post Graduate Student, Department of Oral Medicine and Radiology, Albadar Rural Dental College and Hospital , Gulbarga

***Corresponding author**
Dr Sharashchandra MB
Email: drsharad_004p@yahoo.co.in

Abstract: Ever since its introduction Ultrasound (US) has been evolved remarkably to present features with better promises and reached new horizons, often one such turnover from diagnostic to therapeutic application leads in introduction of micro bubbles. It is a new innovative method with minimal invasive procedure of drug and gene delivery to target sites with a specific targeted ligand on micro bubbles. Micro bubbles are small air filled central core with flexible or rigid shell composed of albumin or lipid or polymer. Micro bubbles on application of US beam rapidly contract and expand in response to pressure change of sound waves. Micro bubbles upon ionization of sufficiently intense pressure will cavitat, sonoporation employs the cavitation of micro bubbles to enhance the delivery of drug and genes, Cavitation with micro bubbles can be used to dissolve the blood clots or drug delivery. Aim of this review is to highlight many such diagnostic and therapeutic application of Ultrasonography mediated micro bubbles.
Keywords: Ultrasound, Micro bubble, Sonoporation, Contrast agents, Drug and Gene delivery, Theranostics

INTRODUCTION

Ultrasound (US) is one of the most advanced imaging modality in the medical and dental field; it uses sound waves for viewing the normal and pathologic conditions of the tissues, its application ranges from first-look examinations of the abdomen and other soft tissues to endo sonography via the esophagus or the female genital tract and intravascular applications [1]. Ultrasound examinations are usually performed without contrast agents and the vascular information derived from color or power Doppler is generally sufficient to find the right diagnosis [1]. However, the development of micro bubbles had considerable impact on the field of diagnostic ultrasound. Microbubbles are minute, gas or lipid-filled spheres have enabled imaging in the kidney, liver, heart, and myocardium [2].

The concept of ultrasound contrast agents (UCA) is based on the inherent physical and acoustical properties of gas-filled micro bubbles within an ultrasonic field. Depending on the magnitude of the incident, ultrasonic wave different scattering behavior occurs, while it is linear for low acoustic pressures and increasing it leads to the occurrence of nonlinear effects such as emission of harmonics and high pressure results in destruction of the bubbles producing highly nonlinear echo signals [3].

Gas-filled micro bubbles have been used as ultrasound contrast agents from some decades. More recently, such micro bubbles have evolved as experimental tools for organ and tissue-specific drug and gene delivery. Micro bubble when sonified with ultrasound near their resonance frequency, micro bubbles oscillate with higher ultrasound energies as oscillation amplitudes increases this leads to micro bubble destruction [4] and lowers the threshold for cavitation, resulting in micro streaming and increased permeability of cell membranes, Interestingly, this mechanism can be used for delivery of drugs or genes into tissue [5].

This review addresses the development of molecular ultrasound imaging and the use of micro

bubbles for thrombolysis, facilitating drug and gene delivery across biologic barriers, and theranostic applications in medical and dental fields.

Sonoporation

Sonoporation refers to the formation of small pores in cell membranes of the micro bubbles by using ultrasound for the transfer of nucleic acid materials [6]. The biological effects of ultrasound are categorized as thermal and non-thermal, non-thermal effects are composed of mechanical perturbation in the vicinity of bubbles and cavitation of bubbles which results in membrane portion [7]. The cavitation bubbles induce cell death or permeability to allow the entry of a drug or genes into the cells. The transfer efficiency depends on ultrasound frequency and intensity [8]. The major advantages of sonoporation are its non-invasiveness and ability to transfer genes and drugs to internal organs without a surgical procedure [9].

Mechanism of sonoporation

Biophysical effects of ultrasound include Cavitation, Radiation pressure, and Micro streaming. Cavitation refers to the growth and collapse of micro bubbles, Radiation pressure is the force in the irradiation field, Micro streaming is the shear forces that exist near the micro bubbles. The formation of cavitation increases with rising ultrasound intensity while the frequency decreases [10] and micro bubble expands and contracts with ultrasound irradiation, these results in micro bubble collapse, the cell membrane is ruptured and a pore is generated [11].

Micro bubble

Micro bubbles are *theranostic* agents; they provide simultaneous and co-localized contrast for imaging (diagnostics) and drug carrying and delivering capacity for targeted therapy [12]. The diameter of a micro bubble is approximately equal to the size of a red blood cell (less than ~10 μm diameter), it allows to display similar rheology in the micro vessels and capillaries throughout the body [13].

The gas core comprises most of the particle volume and provides the mechanism for ultrasound backscatter and drug delivery. Gas bubbles of this size in aqueous media are inherently unstable owing to surface tension effects and therefore require a stabilizing shell. The shell may be composed of surfactants, lipids, proteins, polymers, or a combination of these materials [12].

Biomedical Applications micro bubble

Microbubbles display numerous useful effects when they are insonified by ultrasound [14]. A range of behaviors is available which depend not only on the ultrasound parameters, but also on the micro bubble size and physicochemical properties. Subtle effects such as acoustic backscatter are used for diagnostic imaging. More violent effects such as inertial cavitation can be used for targeted drug delivery [15].

Microbubbles insonified at MHz frequencies produce a variety of effects which may be beneficial for ultrasound imaging or drug delivery. At low acoustic pressures, an insonified micro bubble produces a backscattered echo. The echo can be used to detect and locate the micro bubbles. The micro bubbles can therefore be used as a contrast agent in ultrasound imaging [16].

At acoustic pressures just below the fragmentation threshold, a micro bubbles will undergo dissolution [17] and small volume of the gas core escapes with each cycle. This acoustic dissolution offers a more subtle means of eliminating the contrast signal than fragmentation and may also be useful for drug delivery [18].

A more violent activity may occur at high acoustic pressures and lower frequencies Inertial cavitation occurs after a prolonged expansion phase, where during the subsequent compression phase a strong inrush of water toward the micro bubbles center results in a violent implosion. The implosion emits a shockwave that may be detected by the ultrasound transducer as a broadband signal. The shockwave may also facilitate intercellular and extravascular transport of macromolecules. Asymmetric cavitation near a rigid boundary can result in an involuted jet (water hammer) that impinges on the surface and may further enhance drug delivery [19].

Finally, at low acoustic pressures and at resonance frequency, each cycle of ultrasound results in a net force on the micro bubbles which displaces it away from the transducer (i.e., in the direction of the propagating acoustic wave) [20]. Radiation force may be used to move the micro bubbles from the vessel lumen to the endothelium, thus facilitating ligand-receptor mediated adhesion and targeted drug delivery [21-23].

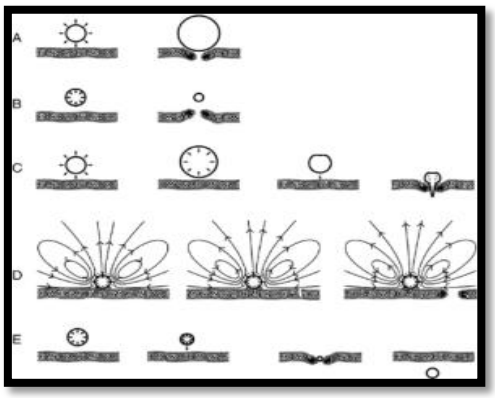

Fig 1: Five mechanisms of pore formation provoked by micro bubbles oscillating under ultrasound.

(A) Push: During its expansion phase, a micro bubble might touch a cell membrane surface, possibly pushing it apart. (B) Pull: During the contraction phase of an oscillating micro bubble, the plasma filling the void left by the contracting bubble might pull the cell membrane towards the micro bubble, possibly disrupting the plasma membrane. (C) Jetting: Jetting is the asymmetric collapse of a bubble, creating a funnel-shaped protrusion through the bubble which is directed towards a boundary. (D) Streaming: If a micro bubble is attached to a cell membrane, the fluid streaming around the oscillating bubbles creates enough shears that could induce cell membrane rupture. (E) Translation: Owing to radiation forces, lipid-encapsulated micro bubbles could translate through cell membrane. The micro bubble may lose part of its shell whilst passing through the cell membrane. Figure adapted from (Delalande *et al*., [24].

Diagnostic application of ultrasound mediated micro-bubble [25-30]

Ultrasound imaging is widely used worldwide principally because it is cheap, easily available and contains no exposure to ionizing radiation. The advent of micro bubbles ultrasound contrast has further increased the diagnostic sensitivity and specificity of this technique thus widening its clinical applications [25]. Ultrasound imaging is clinically established for routine screening examinations of breast, abdomen, neck, and other soft tissues, as well as for therapy monitoring. Microbubbles as vascular contrast agents improve the detection and characterization of cancerous lesions, inflammatory processes, and cardiovascular pathologies [26].

1. To directly visualize the left ventricular and echocardial surface, to determine the left ventricular systolic function,
2. Vascular tree is imaged including aorta, carotid arteries, infra inguinal arteries, bypass and as well as peripheral venous system.
3. Real time imaging of microvascular perfusion including angiogenesis used to image carotid lumen and wall to identify plaque and ulcers,
4. Characterization of focal hepatic lesions and in determining the speed of malignancy during intra-operative period prior to hepatectomy
5. Useful for assessing the splenic trauma and specific focal lesions
6. In detecting pancreatic carcinoma which is usually confused with chronic pancreatitis
7. Used in detecting the bile duct filling defects which is caused by soft tissue masses, to assess the vascularity for identifying infection and carcinoma,
8. To investigate renal artery stenosis and alternative to nephrostograms and it has better imaging than histological examination,
9. In assessing the vascular component of response to systemic anti-tumor and anti angiogenic drug regimes, for preoperative localization of central lymph node after injection contrast enhanced ultrasound (CEUS) micro bubbles.

Therapeutic application of ultrasound mediated micro bubbles

Microbubbles have been proposed as a new vehicle for delivery of drugs and genes. Several properties of micro bubbles make them a promising tool for drug and gene delivery to cells proven by animal models and preclinical studies. [31].

1. High-intensity focused ultrasound combined with microbubbles enhances the therapeutic effects of gemcitabine chemotherapy in a pancreatic cancer xenograft model [32].
2. Low-intensity ultrasound is a useful method to introduce materials into cells due to the sonoporations, on the cell membrane. An oncolytic herpes simplex virus type 1 (HSV-1) can be introduced into oral squamous cell carcinoma (SCC) cells through membrane pores [33].
3. Sonoporation enhances the efficiency of boron phenylalanine (BPA) -mediated Boron neutron capture therapy for oral squamous cell carcinoma. Sonoporation may modulate the micro localization of or boronocaptate sodium (BSH) intraperitoneally in tumors and increase their intracellular levels [34].
4. The combination of Bubble liposomes and ultrasound provides an efficient technique for delivering plasmid DNA into the gingiva. This technique can be applied for the delivery of a variety of therapeutic molecules into target tissue, and may serve as a useful treatment strategy for periodontitis [35].
5. Bubble liposomes can be used in combination with ultrasound to efficiently deliver plasmid DNA into the tongue in vivo. This technique is a highly promising approach for gene delivery into oral tissue [36].
6. Ultrasound promotes the entry of oncolytic HSV-1 into cells. It may be useful to enhance the efficiency of HSV-1 infection in oncolytic biotherapy [37].
7. The cavitation bubbles produced using High-intensity focused ultrasound (HIFU) can be used as a potential method to deliver antibacterial nanoparticles into the dentinal tubules to enhance root canal disinfection [38].
8. The combination of Bubble and ultrasound are advantages specific to spinal gene transfection including minimal invasiveness of simple percutaneous dural puncture, target ability due to the limited access of ultrasound waves through anatomical apertures of the vertebrae, and possible paracrine delivery of therapeutic molecules to the spinal nerve system [39].
9. Ultrasound delivered at higher intensities using either an endovascular vibrating wire or transcutaneously in conjunction with stabilized microbubbles can cause mechanical

fragmentation of thrombus without administration of plasminogen activator, helps in thrombolytic procedures [40].

Advantages

- Real time evaluation of blood flow
- Destruction of micro bubbles allows absolute quantification of tissue perfusion.
- Ultrasonic molecular imaging is safer than molecular imaging modalities because it does not involve radiation.
- Ultrasound is very cost-efficient and widely available compared to other imaging modalities such as MRI (Magnetic Resonance Imaging), PET (Position Emission Tomography), SPECT (Single Photon Emission Computed Tomography).
- Since micro bubbles can generate such strong signals, a lower intravenous dosage is needed.
- Targeting strategies for micro bubbles are versatile and modular. Targeting a new area only entails conjugating a new ligand.
- Active targeting can be increased by acoustic radiation force using a clinical ultrasound imaging system in 2D-mode and 3D-mode.

Disadvantages

- Microbubbles don't last very long in circulation.
- Ultrasound produces more heat as the frequency increases, so the ultrasonic frequency must be Carefully monitored.
- Microbubbles burst at low ultrasound frequencies and at high mechanical indices (MI), which is the measure of the acoustic power output of the ultrasound imaging system. Increasing MI increases image quality, but there are tradeoffs with micro bubbles destruction. Micro bubble destruction could cause local microvasculature ruptures and hemolysis.
- Targeting ligands can be immunogenic, since current targeting ligands used in preclinical experiments are derived from animal culture.
- Low targeted micro bubbles adhesion efficiency, which means a small fraction of injected micro bubbles bind to the area of interest. This is one of the main reasons that targeted contrast-enhanced ultrasound remains in the preclinical development stages.

Contraindication:

The use of micro bubbles contraindicated hypersensitive patients, pregnancy and pediatrics

Conclusion and future prospective:

With the advances in the imaging field, US imaging modality are likely to serve as boon in the field of diagnostic and therapeutic application.US in the field of medicine and dentistry has been increasingly developed and it is gaining more space in dentistry. The use of gas filled micro bubbles with the use of ultrasound is most widely used in medical field with drug/gene delivery. Micro bubble with targeted ligands as a vehicle in transforming drug and gene delivery is an upcoming US modality in dentistry, many researches are undergoing on animal models to better understand its impact on human body, its uses as a therapeutic agents is becoming more wide spread primarily because of its minimal invasive nature of the procedure. Therefore USG appears to be one of the promising therapeutic aids in the near future.

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