INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Review Article

ULTRASONIC MICROBUBBLES - A SUPER SAINT IN PHARMPACEUTICAL TECHNOLOGY

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ABSTRACT

Ultrasonic micro bubbles are the specialized micro spheres containing gas filled contrast agents used for both diagnosis and curative purpose. In general, this is meant for the diagnosis of tumor, cardiac diseases and also in gene therapy. Mainly the ultrasonic micro bubble consists of shell is made up of polymers and gas core which is loaded with drug and heavy gases. In order to float on blood stream, micro bubbles are containing gas core and surfactants necessarily. More over these micro bubbles are having size less than RBC's in such a way that micro bubbles can able to diffuses even into the small capillaries and tissues. Ultrasonic micro bubbles are administrated through I.V route as I.V bolus. After administration it reaches the site of impairment and anchoring on the surface of effected organ. Then after with the help of ultrasonographic instrument which containing ultrasonic fields, from these instrument ultrasonic waves are getting release and break the micro bubbles and drug comes out. These all are coordinated by reticulo endothelial systems which monitor the drug to reach the exact site of action.

Keywords: Targeted Drug Delivery systems, Reticulo-endothelial system.

I. INTRODUCTION

Ultrasonic waves are generally lies above the human audible range (20Hzs-20KHzs) and having characteristic frequencies greater than 20KHzs meant for diagnostic purpose. Commercially available contrast media are gas-filled micro bubbles that are administered intravenously to the systemic circulation. Micro bubbles have a high degree of echogenicity, which is the ability of an object to reflect the ultrasound waves. Ultrasonic micro bubbles containing contrast agents which enables to maintaining the optimum echogenesity to the produced ultrasonic waves. However recent researches concluded that incorporation of ligands in the micro bubble urges ideal results in the diagnosis of cardio- vascular diseases and also in gene therapy gives the good quality images after administration of drug into systemic circulation through I.V bolus[1,2]. By this way with the help of Ligands good improved imaging of soft tissues and organs are possible[3,4].

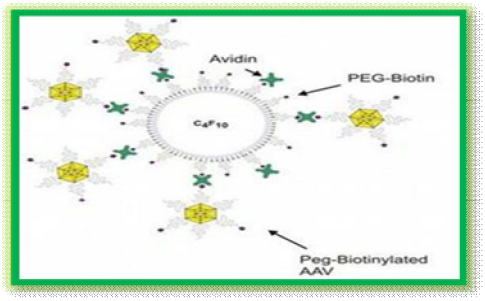


Fig. 1: Ligands containing micro bubble for diagnosis of cardiac diseases

WHO and U.S FDA stated that ultrasonic micro bubble technology is safe, and enables reliable results are observed, without affecting the functions of the organs which gives the

perfect images[5,6]. After entering of the micro bubbles inside the body, these are specifically binds to the impairment organ. Then after ultrasonic waves are released, this are reached to the bubble and shows the breakdown of the bubble. Because of this ultrasonic back scatter is produced due to the higher acoustic energy difference between the micro bubble and the surrounding tissues[35]. In this technique 1-10 MHz frequency is tuned for diagnosis purpose[7].

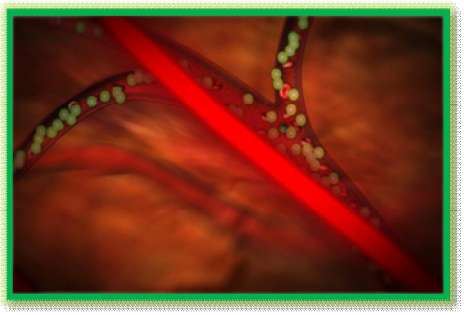


Fig. 2: Ultrasonic micro bubbles in systemic stream

II. MICRO BUBBLE CONTRAST AGENTS

1. Ideal objectives of micro bubble system.
2. Specifications of micro bubble.
3. Different echo contrast agents used in ultrasonic micro bubbles.

1. Ideal objectives of micro bubble system

- Micro bubbles must synchronize the compatibility action with immune system.
- It should provide substantive site specific character and increased safety margin during process.
- It didn't enable tactlessness during diagnosis and treatment of diseases.
- It must be non irritant and reduced systemic side effects more over it facilitates improved therapy.
- Ultrasonic micro bubble system must propagate good therapeutic efficacy with lower frequency of dose.
- It should obligate wide range of acoustic energies and good echogenicity to the ultrasonic field.
- Shell must having the property to obstruct the leakage of drug from the micro bubble.
- More over it can able to confide magnificent sonogram and images.

2. Micro bubble Specifications

Mainly micro bubble containing i. shell
ii. Gas core

i. Micro bubble shell

Micro bubble shell is responsible for the echogenic character by enclosing the outer part of the micro bubble system, and also it impart good resilience power to resort the micro bubble even into the small capillaries. Micro bubble shell is made up of hydrophillic substances to reduce the residence time in the systemic pool as well as promots the biocompatibility with immune system by facilitating the micro bubble implicate to immune system fairly. Shell consists of polymers, albumins, galactose and lipids which are intended for the compatability action with the immune system [8, 9]. However shell executs wide range of flexibility to the optimised acoustic energy, and it helps the micro bubble exceptionally stayed for prolonged time in circulation [8,9].

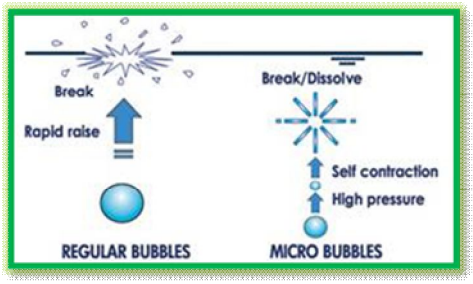


Fig. 3: Advantage of micro bubble over regular bubble

ii. Micro bubble gas core

Gas core is having significant role in the ultrasonic micro bubble technology, because gas core is loading with both drug and gases. Gas core consists of gases like Perfluorocarbans, Nitrogen and some heavy gases [35]. If the prepared micro bubble containing heavy gases it sanctify the prolonged systemic circulation of micro bubble. In the systemic circulation gas core helps in, meritoriously maintaining the echogenic character to the micro bubble and also it prevents the leakage of gas and drug from the micro bubble [9]. Upon reinforce the ultrasonic field on micro bubble a perfect gas core gives good sonogram and echogenicity.

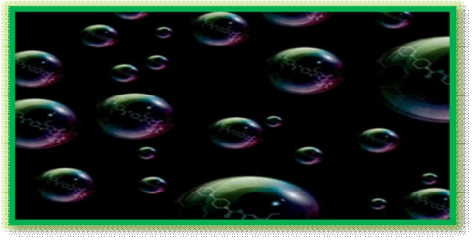


Fig. 4: Classical Diagrammatic representation of Gas core containing micro bubbles

Table 1: Different echo contrast agents used in Ultrasonic micro bubble technology[34]

Agent	Bubble size mean(µm)	Gas	Shell composition	Behavior	Micro bubble destruction
Albunex	4.5	Air	Albumin	Intravascular tracer	Sensitive
Levovist	2-3	Air	None-bubble adhere to galactose micro particle	Intravascular tracer	Sensitive
Echogen	2-5	Perfluoropentane	Stabilized surfactant	Deposit agent	Resistant
Sonogen	2-5	Perfluoropentane	Anionically charged surfactant	Deposit agent	Resistant
Opison	4.7	Perfluoropentane	Albumin	Intravascular tracer	Sensitive
Definity	1.5	Perfluoropentane	Phospholipids	Intravascular tracer	Sensitive
Imagent	5	Perfluoropentane	Perfluoropentane	Intravascular tracer	Sensitive
Sonovue	2.5	Sulfur hexafluoride	Phospholipids	Intravascular tracer	Sensitive
PB 127	4.0	Nitrogen	Biodegradable polymer bilayer	Intravascular tracer	Designable
NC100100	3.4	Unspecified	Unknown	Intravascular tracer	Sensitive
AI-700	2	Perfluoropentane	Synthetic polymer	Intravascular tracer	Resistant

III. ULTRASONOGRAPHIC INSTRUMENT

Fig. 5: The Ultrasonographic instrumenet
Instrumentation

It consists of
1. Display monitor.
2. Control setting system.
3. Transducer.
 i. Piezoelectric transducer.[Or]
 ii. Linear array transducer.
4. Frame grabber.
5. Doppler unit.
i. Color Doppler.
ii. Power Doppler.

1. Display monitor system

Display monitor system is intended for visualization of ultrasonic micro bubbles which are present in the body, more over display system helpful in the locating the micro bubbles where these are anchoring to the site of impairment. Hence display monitor system patronizes the status of the disease by obligating the images.

2. Control setting system

Control setting system is nominated to set the accurate frequency of the ultrasonic waves during process. With the help of this adjust the frequency range depending upon the organ subjected for the ultrasonication.

For suppose we want to subject the superficial organs to ultrasonication we can optimize the frequency range in between 7-18 MHz with the help of control setting system

And in the case of deeper structures 1-6 MHz frequency is sufficient. For Targeted system 6-12 MHz frequency is required. For Untargeted system 14-18 MHz frequency should be maintained.

3. Transducer

This is the specialized equipment used to pass the ultrasonic waves into the body through it. In general transducers are 2 types.

a. Piezo electric transducer.
b. Liner array transducer.

a. Piezo electric transducer

Piezo electric transducer is one type of transducer helps in the generation and conduction of ultrasonic waves in older treatment. It generates good and strong ultrasonic field and the optimum frequency

range conducted by Piezo electric transducer lies in between 2-18 MHz's.

b. Linear Array transducer

Fig. 6: Linear Array Transducer.

Now a day we use this type of transducer in medical ultrasonography for the purpose of generating and conducting ultrasonic waves. It generates good and strength beam of ultrasonic waves. Easy handling of linear array transducer is notified. Optimizing range lies between 7-14 MHz's in linear array transducer.

4. Frame grabber

Frame grabber is helpful in the displaying the image. Coming to the main function of the frame grabber is displaying, capturing, and broad casting through monitor. It enables video signals, digitalized displaying and capturing modes [10].

5. Doppler instrument

Doppler instrument is useful to detect the micro bubble compatibility and measuring the frequency shift of the sample. It also controls the speed of the ultrasonic waves. This is the instrument responsible for creating the difference in the acoustic energies and formation of ultrasonic back scatter responsible for breakdown of micro bubbles.It is of 2 type's a. Color Doppler. b. Power Doppler.

a. Color Doppler

Color Doppler is a directional Doppler. This allows the uniform direction of ultrasonic waves into the body. Depending upon this prospective image is formed. Some laboratories form the image at near red shift, i.e. favors higher echo side. And some labs prefers blue shift for image, i.e. at lower echo side, because of this confusion now days color Doppler is performed depending on the normal and abnormal blood flows in human body [11-14].

b. Power Doppler

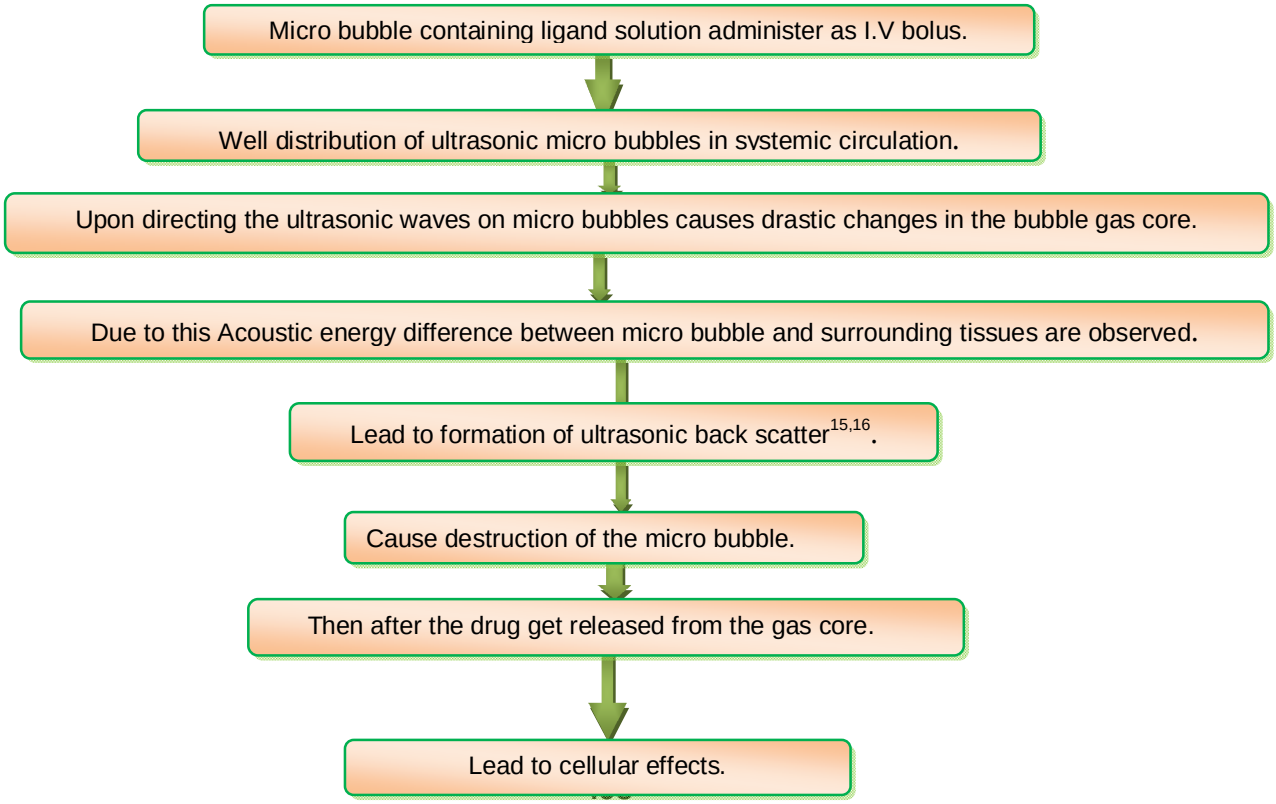
The name itself suggesting that power Doppler shows the non-uniform and non-directional Doppler signals. Depending upon the velocity of waves it is random in nature. Due to this power Doppler facilitated images formed also random in nature.

IV. WORKING MECHANISM

1. Pin point view of working mechanism.
2. Types of working mechanism.

A. Targeted micro bubble system
B. Untargeted micro bubble system.

1. Pin point view of working mechanism



2. Working mechanism of ultrasonic micro bubble technology

Mainly 2 types of mechanisms are there

A. Targeted ultrasonic micro bubbles system.

B. Untargeted ultrasonic micro bubble system.

A. Targeted ultrasonic micro bubble system

The name itself suggesting that we target the specified organ of interest for both diagnosis and curative purpose. Here we selecting an individual impairment organ and capturing the high quality images for that organ

.

Micro bubble containing ligands are administered as small amount of I.V bolus.

Then micro bubbles are entering into the systemic circulation.

After that these micro bubbles are statistically adhere to the site of impairment organ.

Then ultrasonic waves are guided along the axis of interest into the body.

Then after differences in the echogenesity and acoustic energies between the micro bubbles and surrounding tissue causes ultrasonic back scatter[15,16].

Due to this micro bubble shell become ruptured.

Because of this gas core containing medicament releases out.

Lead to cellular effects.

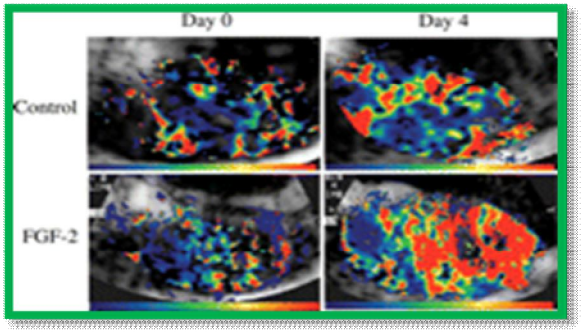
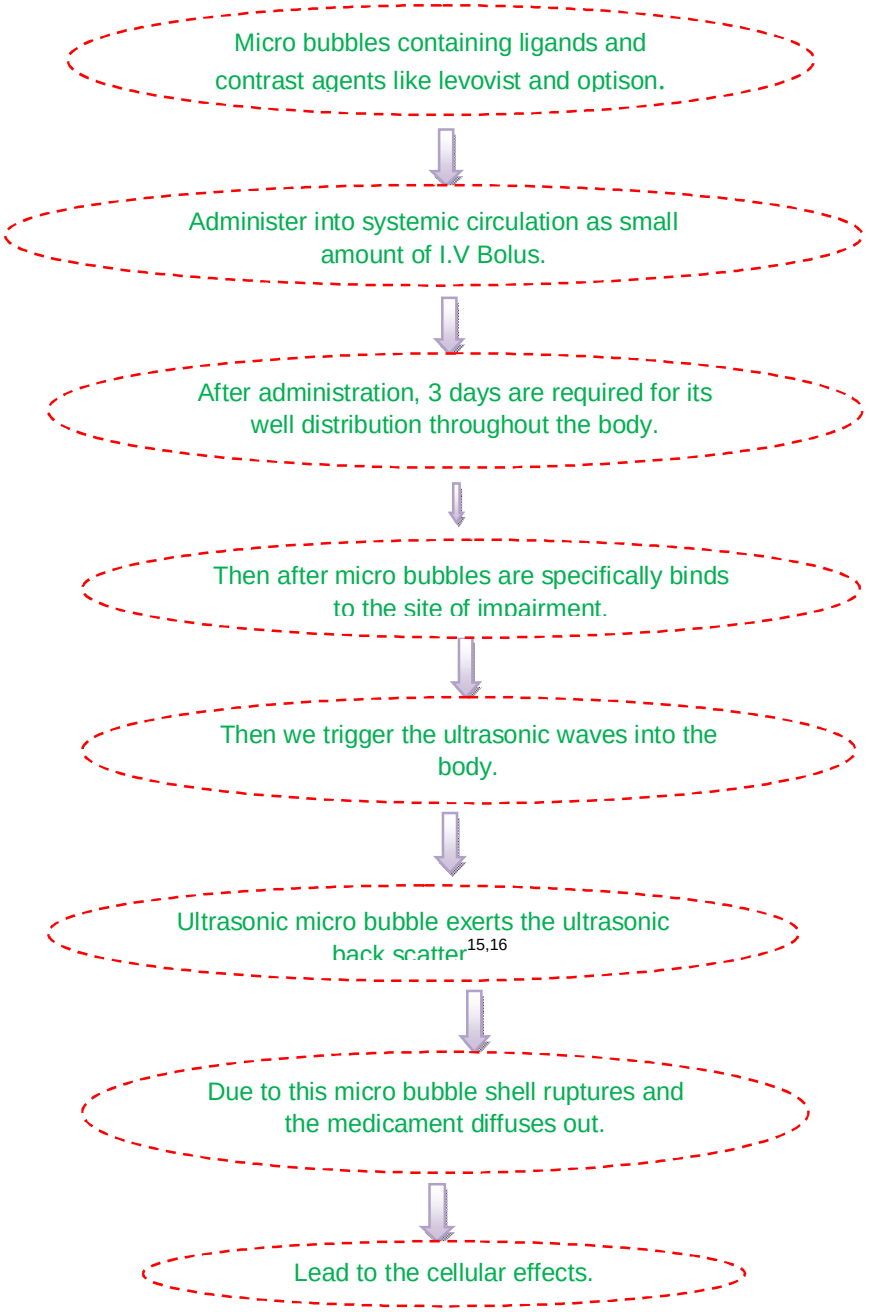


Fig. 7: Targeted micro bubble system consists of Liver imaging

Mainly targeted ultrasonic micro bubble system is used for the diagnosis of kidneys, heart and some localized tumor impairments. In this the ultrasonic wave frequencies lies in between 7-12 MHz's.

B. Untargeted ultrasonic micro bubble system

Untargeted ultrasonic micro bubble system is used in the treatment of blood cancer and somehow in chronic malignant tumors. In this system we didn't targeting the specified organ, depending upon these criteria we choose the contrast agents as optison or levovist along with ligands. For this purpose we will closely monitor the patient at least 3 days. Because it is the necessary time required for its better distribution of micro bubble throughout the body.



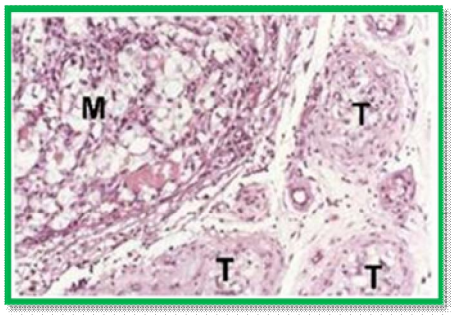


Fig. 8: Untargeted micro bubble system consists of imaging of Blood cancer
(untargeted ultrasonic micro bubble system the optimized range lies between 14-18 MHz's)

V. Brief Discussion About Ultrasonic Micro Bubble Elimination From Body

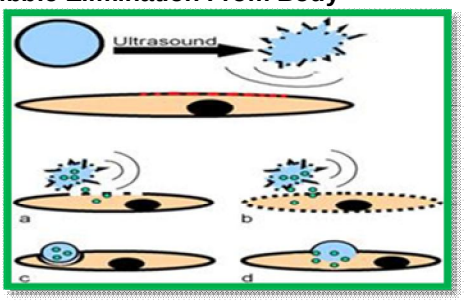


Fig. 9: Destruction of micro bubble by ultrasound waves [Ultrasonic Micro bubbles: A New Vista In Drug Delivery And Medical Imaging by manikiran]

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

1) Ultrasonic micro bubbles are bio-compatible and bio-degradable.
2) After exerting its action, these are excreted from the body itself as urine, and also in the form of sweat.
3) In case some micro bubbles are didn't show breaking and still stay in systemic pool, these micro bubbles not execute any poisonous effects to the individual. And they get degraded with in short period of time.

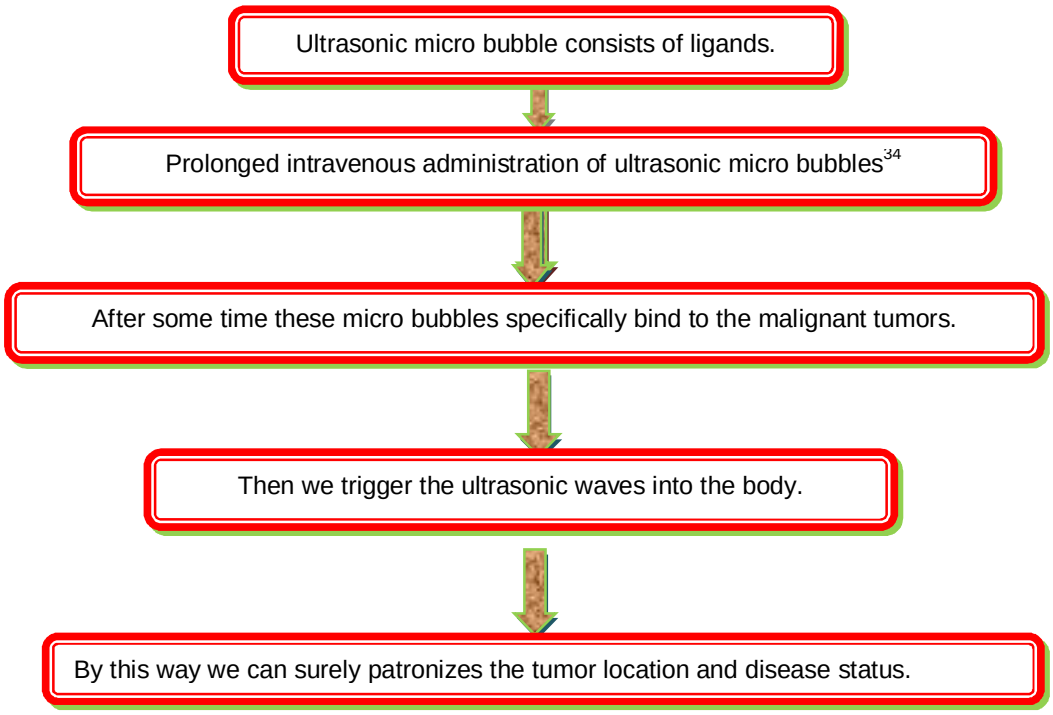
VI. ADVANTAGES

Under this discuss about
1. Diagnostic advantages.
2. Therapeutic advantages.
3. General advantages.

1. Diagnostic advantage

It includes mainly
A. Imaging the Tumor.
B. Imaging the Liver.
C. Imaging the Heart.
D. Gene delivery.

A. Imaging the Tumor



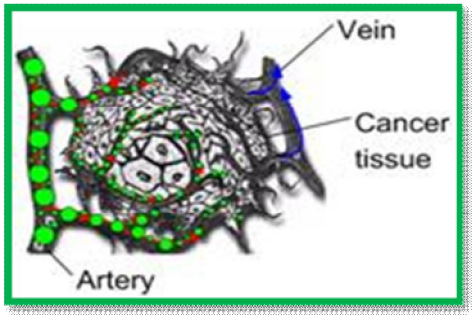


Fig. 10: Schematic diagram of a micro bubble used for cancer

- Tumor diagnosis is possible like this way in both Targeted and Untargeted micro bubble system.

- **B. Imaging the Liver**

However this mechanism is not clear so far.

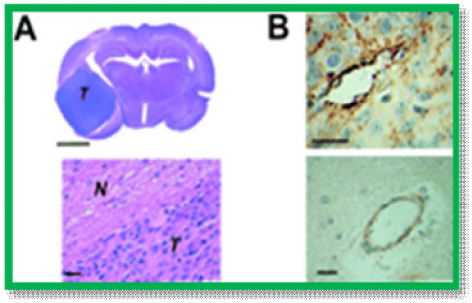


Fig. 11: Ultrasonic micro bubble imaging of Liver

Micro bubbles containing ligands and contrast agents.

Administration of micro bubbles into systemic circulation as I.V bolus.

Here in this mechanism micro bubbles are probably detected by Reticulo endothelial system.

Then after this micro bubbles are taken up by the liver and spleen[17].

Then these micro bubbles are binds to the damaged sites of the liver.

Then we trigger the ultrasonic waves into the body micro bubbles are burst out.

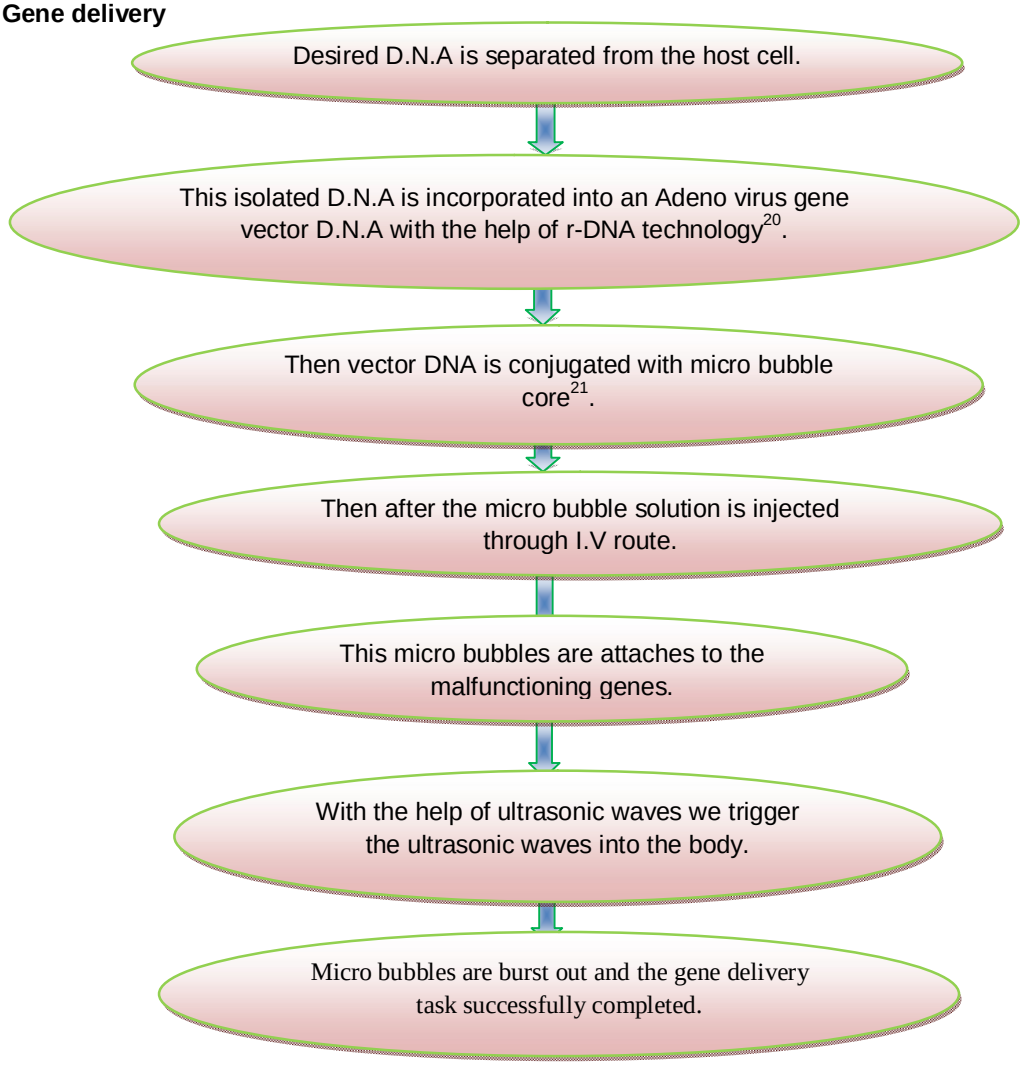
Here we can diagnosis of the disease is by identifying where the population of micro bubble is more.

Like this way imaging of liver is carried on.

C. Imaging the Heart

Mainly this technique is reinforced in the treatment and diagnosis of atherosclerosis and contiguous treatment in the coronary artery diseases. We can provide constitutional diagnosis in the Valvular sternosis [18], and also in the deep vein thrombosis [19]. During heart imaging we can rivet the abnormalities in the wall motion and blood flow and valves function in heart. More over these micro bubbles are bind to the site of impairment i.e. atheroma as per mechanism of action. After that as usually we navigating the ultrasonic beam, due to this bubble get break down and the drug is immediately releases from the micro bubble and imaging of heart is propagated.

D. Gene delivery



2. Therapeutic advantages

- Ultrasonic micro bubbles are useful in cancer, atherosclerosis and angiogenesis treatment.
- Diseases like inflammation, thrombus, and lymph nodes are treated with different ligands like ICAM-1, TNF-alpha[8, 22]
- In the autoimmune diseases like myasthenia gravis and rheumatism we use antibodies p-selection, Arg-Gly-Asp, GpIIb/IIIa [8, 22].
- Micro bubbles are mainly useful in the gene delivery, because viral vectors uses are limited due to its unwanted immune responses[23].
- Gene transfer is done by using contrast agents like albunex, optison and levovist are incorporated in ultrasonic micro bubbles[23].
- Leucocytes having more adhesion capacities due to dual ligand selectin integrin cell arrest system. This shows kinetically improved adhesion[24].
- In this contrast agents bind to ligands, with techniques such as dual-ligand targeting of distinct receptors to polymer microspheres[25], and biomimcry of the leukocyte's selectin-integrin cell arrest system[26], helps in increased adhesion efficiency, but this technique performance poor in targeted system[24,27].
- Ultrasonic micro bubbles are used in the treatment of cardiac diseases like angina pectoris, myocardial infarction, and some gene linked diseases and also in the treatment of familiar hypercholestymia[28].

3. General advantages

- Ultra sonic micro bubble technology is safe and very effective according to the WHO and US-FDA[5].
- With the help of ultrasonic micro bubbles we can revokes high quality images with cost effective manner[29, 30].
- It is very safer than the molecular imaging modalities like MRI (Magnetic Resonance Imaging.), PET (Positron Emission Tomography), SPECT (Single Proton Emission Computed Tomography)[31].
- Generally molecular modalities releases radiation during processing, but in case of ultrasonic micro bubble technology there is no matter of radiation.
- In MRI technology MRI contrast agents are used, these are not effective as ultrasonic micro bubbles[30].
- This ultrasonic micro bubble technology is of very less cost when compared with MRI, PET, and SPECT and also widely available[30].
- Here in this technique we use ligands which are the part of ultrasonic micro bubbles urges good therapeutic results in comparison with other molecular modalities. This yields information that ultra sonic micro bubble technology is a versatile technique.
- It will' not produce any sort of noise or cell damage during processing, but other molecular modalities causes cell damage due to radiation and these are very noisy.
- Ultrasonic micro bubbles are also used as liposome by coating shell with lipid polymers in drug delivery.
- More over it requires fewer doses for imaging as well as therapeutic results [30].
- Beside its use to assess cardiac function and perfusion it is also used as an enhancer of the images of prostate, liver, kidney and other organs[32].

VII. Disadvantages

- Skilled persons are required for its good navigation.
- Frequency of ultrasonic waves should be maintained in the optimized range, because when the upper limit causes micro vascular rupture and at the same time causes cell damage. At lower limit it will not show any therapeutic responses[33].
- Micro bubbles will not have prolonged circulation capacity due to less residence time in systemic pool even though we use PEG[30].
- These micro bubbles are easily taken up by liver and spleen where destruction of micro bubbles is possible without showing any effect in the body.
- Even though the frequency range is maintained within the normal range the micro bubbles seldom breaks sometimes.
- In case of targeted micro bubble system these micro bubbles are bound to the normal healthy cells. So, during that time it causes surrounding healthy cell damage. This is the reason it is in the pre-clinical stage still[33].
- In targeted system micro bubbles containing ligands may be immunogenic. That's way it is in pre-clinical stage[33].
- Low targeted micro bubbles binding efficiency is poor; this is another reason it is in the pre-clinical development stage[22].

VIII. CONCLUSION

The ultrasonic micro bubbles technology having greatest flexibility as well as versatility in medical field, for the diagnosis and treatment of malignant tumors, cardiac diseases and also in gene transfer. Now days this technique is widely used across the world because of its safety and affectivity concern. According to WHO and US-FDA it is the one of the leading technique for the treatment of cancer and gene malfunctioning diseases. More over it poses very few disadvantages and these are negotiates compare with enormous focusing advantages of ultrasonic micro bubble technology. In such way that this technique is concluded as super saint in pharmaceutical and medical fields because ultrasonic micro bubbles utility increases day by day.

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