Chapter

Using Microbubbles as Targeted Drug Delivery to Improve AIDS

Harsha Virsingh Sonaye, Rafik Yakub Shaikh and Chandrashekhar A. Doifode

Abstract

No preventive vaccines are available for the treatment of AIDS. To improve therapy, combinational antiretroviral drugs are given; however some patients develop resistance to particular combinational drug. Microbubble-mediated drug delivery technology solves the problem by reducing systemic dose and toxicity. Microbubbles are bubbles smaller than one millimeter in diameter but larger than one micrometer. The general composition of microbubble is gas core. The mechanism of microbubbles through which its delivery increases is sonoporation, the formation of openings in the vasculature, induced by ultrasound-triggered oscillations and destruction of microbubbles. Rapid isolation strategy of CD4+ cells is mixing blood and glass microbubbles which then bind with the specific target cells to the microbubble carrying specific antibodies on their surface. The target cells will spontaneously float to the top of the blood vial and can be quickly separated. The microbubbles are particularly used in the diagnosis of AIDS because of their cell isolation techniques which is rapid and inexpensive and their small size to pass through capillary for perfusion in tissue This review demonstrates the problems with the current treatment of the disease and shed light on the remarkable potential of microbubbles to provide more effective treatment and prevention for HIV/AIDS by advancing antiretroviral therapy, gene therapy, immunotherapy, vaccinology, and microbicides.

Keywords: microbubbles, HIV/AIDS, target drug delivery

1. Introduction

The recent advanced methods of noninvasive delivery of therapeutic agents are effective in gene therapy and molecular biology. Besides the well-known application of microbubbles have been demonstrated an effective technique for targeted delivery of drugs and genes and is also used as contrast agents for diagnostic ultrasound [1–6]. A schematic structure of the biomedical microbubble is shown in **Figure 1**. The size of microbubbles is larger than micrometer but smaller than one hundredth of millimeter in diameter which is equal to the size of red blood cell. Because of its smaller size, it can pass in the microvessels and capillaries throughout the body. In aqueous environment, the microbubbles are unstable and show surface tension effect because of this properly it require the shell and filling material. The gas core of microbubbles gets stabilized by lipid, protein, and polymers [7, 8]. In water microbubbles are miniature gas bubbles of less than 50 microns diameter.

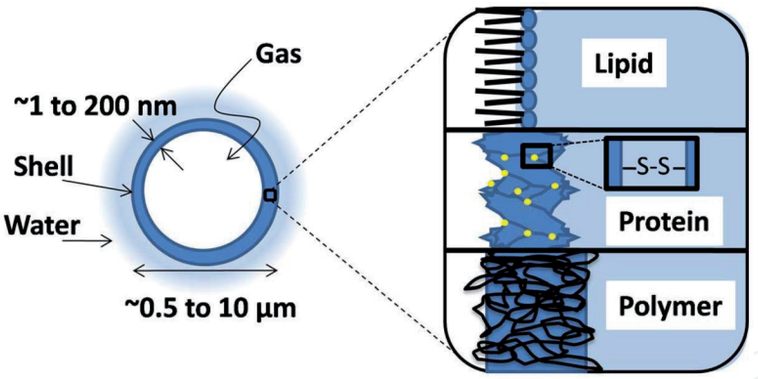

Figure 1.
Illustration describe various shell compositions of microbubbles. The diameter between 0.5 and 10 µm is applied for biomedical use so that it can pass through the capillary of the lung. Microbubbles compose of total particle volume which act as single chamber so that the shell of the microbubble separate encapsulated gas and the surrounding aqueous medium by using various shell materials like lipid with thickness~3 nm thick, protein having 15–20 nm thick and polymer of 100–200 nm thick. Hydrophobic and Vander Waals interactions binds the lipid molecule together and by covalent disulphide bonding the protein molecules get cross-linked so that the formation of bulk like material.

It mostly contains oxygen or air and remains suspended in the water for an extended period. The gas present in the microbubbles dissolves into the water, and the bubble disappears. Incorporation of drug in microbubble includes (1) binding of drug to microbubble shell and (2) attachment of drug at specific site of ligand. In ultrasound-mediated microbubbles, application of high intensity ultra sound can rupture capillary blood vessels resulting in deposit of protein and genetic material into the tissue, ultrasonic rupture of microvessels with diameter 7 µm. Ultrasound forms pores in the membrane of shell. Ultrasound microbubble causes transient hole in the cell surface resulting in rapid translocation of plasmid DNA from the outside to cytoplasm. Low-intensity ultrasound microbubble (0.6 W/cm^2) caused enhanced drug delivery [55]. Microbubbles are usually injected intravenously which is a safe process as compared to the use of conventional method like magnetic resonance imaging and radiography. Microbubble is used in the medical field as diagnostic aids to scan the various organs of the body, and recently they are being proposed to be used as drug or gene carriers and also for treatment in cancer therapy. It is also used to improve the fermentation of soil, to increase the hydroponic plant growth, to increase the aquaculture productivity, and to improve the quality of water, in sewage treatment.

2. Compositions and physicochemical properties of microbubbles

2.1 Protein as stabilizing agent in formation of microbubbles

Albumin-shelled microbubbles were a pioneering formulation used in contrast ultrasound imaging. For perfusion in capillary and microvessels, albumin-shelled microbubbles are very effective. The size of albumin-shelled microbubbles ranges from 1 to 15 µm in diameter in 7 × 10^8 microbubbles/mL which is stable for 2 years. To formulate albumin-coated microbubbles by sonication method, 5% w/v human serum albumin with air is required and encapsulated within 15 nm thick shell of aggregated albumin. For better encapsulation process, the denaturation of albumin by heating is essential [9, 10]. The albumin shell is held together through disulfide bonds between cysteine residues formed during cavitation [11]. Covalent cross-linking may explain the relative rigidity of albumin shells observed during ultrasonic insonification [12]. Apart from albumin, several proteins are used to coat microbubbles.

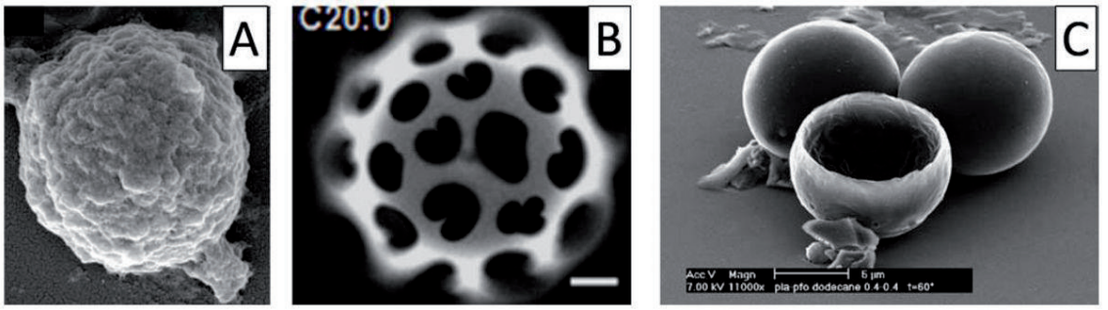

Figure 2.
Microbubble shell morphologies. (A) A lysozyme protein microbubble imaged with SEM (Calaveri et al. (13)). The microbubble diameter is roughly 1 μm. (B) A diC20:0 phospholipid microbubble imaged with fluorescence microscopy taken from Borden et al. Scale bar denotes 20 μm. (C) A PLA-PFO polymer microbubble imaged with SEM.

The proteins which are amphipathic in nature are highly surface-active. In most of the proteins, the disulfide bridge between two thiol groups is present. Cavalieri and co-workers prepared microbubbles by using lysozyme which retain their enzymatic activity for several months and found to be stable [13]. Korpanty et al. [14] developed microbubble by incorporating avidin into albumin shell. **Figure 2A** illustrates targeting vascular endothelium in biotin-mediated coupling of antibodies.

2.2 Surfactant as stabilizing agent in formation of microbubbles

SPAN-40 and TWEEN-40 are used as stabilizing agent in the preparation of microbubbles [15, 16]. For the formation of stable microbubbles, the SPAN/TWEEN solution was sonicated in the presence of air. For maximum film stability, a Langmuir trough was used in the ratio of SPAN to TWEEN (roughly 1:1). By using sonicated microbubbles, modified surfactant was formed which was more stable film due to higher collapse pressure on the Langmuir trough [16]. Dressaire et al. recently reported stable microbubbles formed from a blending process at 70°C in 75 wt% glucose syrup, sucrose stearate (mono- and di-ester) formed [17].

2.3 Lipid as stabilizing agent in the formation of microbubbles

For biomedical imaging and drug delivery, lipid-coated microbubbles are one of the most interesting and useful formulations. The lipid shell is inspired by nature, as stable microbubbles found ubiquitously in the oceans and freshwaters of earth are known to be stabilized by acyl lipids and glycoproteins [18].

During ultrasound and sonication technique, the lipid molecules which are held together by weak physical forces form the microbubble shell having property of expansion and compression without chain entanglement. Lipid-coated microbubbles therefore reduce the damping effect on resonance and reseal around the gas core during fragmentation process [12]. Thus, the lipid-coated microbubble itself is a versatile platform technology. An example of lipid microbubble is shown in **Figure 2B**, which depicts heterogeneity and phase separation of phosphatidylcholine and lipopolymers that are typically used to stabilize lipid microbubble [19].

2.4 Polymer as a stabilizing agent in the formation of microbubbles

The term, "polymer microbubble" typically refers to a special class of microbubbles that are stabilized by a thick shell comprising cross-linked or entangled polymeric species. Polymer shells are more resistant to expansion and compression;

therefore during drug delivery, it reduces echogenicity. During insonification polymer microbubbles release gas core which was unstable and rapidly dissolved [8, 20]. In 1990 a new polymer-shelled microbubble was reported by Wheatley et al. [21] in which the shell was formed by the ionotropic gelation of alginate. By using concentric jells of air and alginate solution, the microbubbles were prepared that was sprayed into a reservoir. On plunging into the calcium solution, the alginate was absorbed by the gas/liquid interface and was hardened. To increase the microbubble yield, sonicate the solution prior to spraying. By using the flow rate of air around the syringe needle, microbubble size was primarily determined. The diameters of microbubbles ranged between 30 and 40 μm and were therefore too large for intravenous administration. In 1997, Bjerknes et al. [22] introduced a method for making microbubbles using an emulsification—solvent evaporation method—encapsulated by a proprietary double-ester polymer with ethylidene units. The polymer microbubbles had a diameter ranging from 1 to 20 μm diameter. Optical microscopy and cryogenic transmission electron microscopy (cryo-TEM) were used for the determination of elongated, crumpled shapes of the microbubbles. The polymer shell was typically 150–200 nm thick. Acoustic tests determine a dose-dependent increase in acoustic attenuation. In 1999, Nayaran and Wheatley describe the preparation of microbubbles by using the biodegradable copolymer poly(D,L-lactide-co-glycolide) (PLGA). By using a volatile solid core, the microspheres were made hollow which could be sublimed. Manipulation of the solution viscosity, polydispersity, and shearing rate microbubble size was controlled. The size distribution ranged from 2 to 20 μm diameter. After incubation in serum the zeta potential of the microbubbles became less negative. In 2005, Cui et al. [23] reported the fabrication of PLGA microbubbles by using a double emulsion, solvent evaporation method. Coulter counter determines the size ranges between 1 and 2 μm diameter. Scanning electron microscopy (SEM) is used to study surface of particles so that the smooth surfaces, visible pores, or cavities can be explained. Confocal scanning microscopy explains internal morphology so that a single hollow core to a more honeycomb structure could be explained depending on the emulsification conditions. In 2005, Cavalieri et al. [24] determined a method of coating microbubbles by using PVA. In this case chemical cross-linking of PVA with microbubbles occurs at the air/water interface with a speed of 8000 rpm, so the mean diameter was approximately 6 ± 1 μm. By decreasing the operating temperature from room conditions to 4°C, the shell thickness could be decreased from 0.9 to 0.7 μm. PVA microbubbles enhance the shelf life of microbubbles by several months. This also increases the incorporation capability of hydrophobic drug and targeting ligand in microbubbles. Bohmer et al. [25] in 2006 used inkjet printing and developed a new technique for the preparation of polymer microbubbles. In this method they injected copolymer polyperfluorooctyl oxycaronyl-poly (lactic acid) (PLA-PFO) having a diameter of 4–5 μm as an organic phase into the aqueous solution (**Figure 2C**).

2.5 Microbubbles used as polyelectrolyte multilayer shells

Polyelectrolyte multilayer (PEM) is a modified type of polymer surfactant shell for the formulation of perforated microbubbles. These microbubbles are coated with charged surfactant which acts as a substrate. To absorb oppositely charged polymer, the layer by layer assembly technique is used [26]. Borden et al. [27] used trimethylammonium propane (TAP) which is a phospholipid containing the cationic head group for creating PEM microbubble where TAP serves as shell. Lentacker et al. [28] described multilayer microbubble. The coating material is DNA and PAH which protect the DNA from enzymatic degradation.

3. Types

1. Perfluorocarbon-filled microbubble, which is stable for circulating in the vascular system as blood pool act as carrier.

2. Ultrasound microbubble, when applied over skin surface where it bursts and releases drug. It is use in low concentration. It also increases therapeutic index. It is advantageous for those drugs which have hazardous and toxic effect.

3. Albumin-encapsulated microbubble, which adheres to vessel walls.

4. Phospholipid-coated microbubble, which has a high affinity for chemotherapeutic drugs [55].

4. Applications

1. Microbubbles increase adherence to damaged vascular endothelium. As the viral proteins obtain in immune response within target tissue the use of viral vector is limited in gene therapy. It has been seen that viral vector causes an intense inflammatory activation of endothelial cells [55].

2. Ultrasound when applied over the skin surface bursts the microbubbles which causes localized release of drug [29–32]. This technique require lower concentration of drug systemically and the concentration of drug only where it is needed therefore the therapeutic index may be increased which is advantageous in case of drug with hazardous systemic side such as cytotoxic agents [33].

3. In diagnostic ultrasound, microbubbles create an acoustic impedance mismatch between fluids and tissues to increase reflection of sound which is used in radiology and cardiology for the detection of perfusion and characterization of tissues. Microbubbles not only increase reflection of sound, they also increase the absorption of sonic energy [34].

4. Ultrasound to contrast agents creates extravasation points in skeletal muscle capillaries. High-intensity ultrasound can rupture capillary vessel resulting in the deposition of protein and genetic material into the tissues. Only a small capillary rupture was required to deliver large quantities of colloidal particles to the muscles [35].

5. Ultrasound increases the transmembrane current as a direct result of membrane resistance due to pore formation [36].

6. Ultrasound-induced cavitation may then be used to destabilize the carriers and affect local drug release. Applications of sonodynamic therapy may include tumor ablation and treating vascular disease such as atherosclerotic plaques. To make targeted microbubbles, targeting ligands were developed and were called bioconjugates suitable for incorporation into membranes stabilizing microbubbles.

7. The anchor locks the bioconjugate into the membrane surrounding the microbubble, and the linker gives the peptide-based targeting ligand enough motional freedom to bind to its target (see **Figure 3**). Thrombus-specific

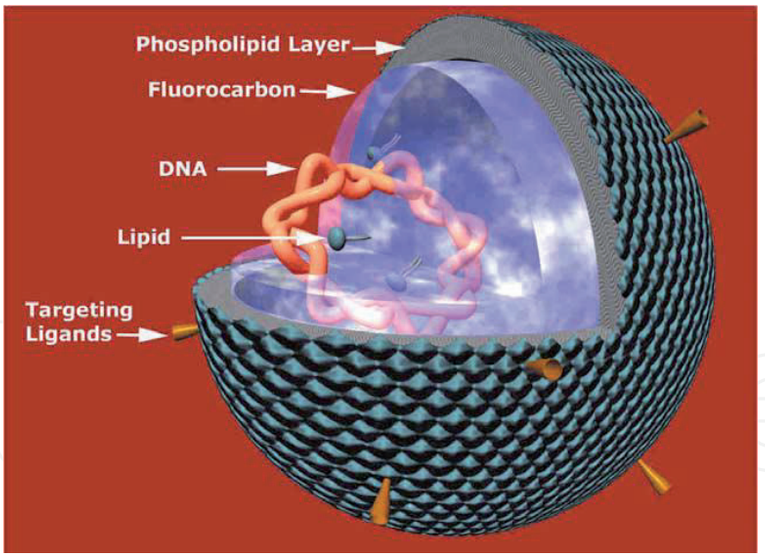

Figure 3.
Liquid perfluorocarbon gene carrier.

peptides, directed to the activated GP2B3A receptor of platelets, were evaluated for affinity to bind to activated platelets by testing for the inhibition of platelet aggregation.

In **Figure 3** the outer surface is stabilized by amphipathic lipid. Targeting ligands have been incorporated onto the head groups of the lipids. The genetic material is stabilized by cationic lipids. Electron microscopy studies have shown that the DNA is condensed as an electron-dense granule within the center of the nanoparticle. The diameter of these particles is about 100–200 nm [37]. There are several advantages to lipid shells. At the air-Space minimized, the phopspholipid's hydrophobic acyl chains face the phopspholipid's gas, and hydrophilic head groups face the water. Thus the monolayer will form around a newly trained gas bubble. Saturated diacyl phospholipids have very low surface tension below phase transition temperature. This is essential as surface tension at the curved interface induces a Laplace overpressure, thus forcing the gas core to dissolve [8]. The microbubble stabilizes at low tension which is achieved by the lipid monolayer [38]. Monolayers of lipids are highly cohesive and form solid-like character because of the attractive hydrophobic interaction between the tightly packed acyl chains and van der Waals [39]. These effect can be effective because the stability of microbubbles during sonication is not dependent on superoxide formation to facilitate disulfide bridging, as is the case with proteins. Therefore, as recently described by Stride and Edirisinghe, lipids are suitable for a variety of manufacturing techniques apart from sonication [40].

In the absence of ultrasound, if the adenovirus was administered with microbubbles using the same model, the author confirmed that plasmid transgene expression can be directed to the heart, with an even higher specificity than viral vectors and that this expression can be regulated by repeated treatments [41]. Lu et al. [42] have also shown that albumin-coated microbubbles significantly improved transgene expression in skeletal muscle of mice, even in the absence of ultrasound.

5. Mechanisms for target drug delivery using microbubbles

Based on the cavitation of microbubbles, two possible strategies for delivering drugs and genes with microbubbles are emerging: the first consists on the ultrasound-mediated microbubble destruction and the second is the direct delivery

of substances bound to microbubbles in the absence of ultrasound. Different drugs and genes can be integrated into the ultrasound contrast agent such as perfluorocarbon-filled albumin microbubbles which actively bind proteins and synthetic oligonucleotides [43]. Microbubbles can directly take up genetic material, such as plasmids, adenovirus, and phospholipid-coated microbubbles as these have high affinity for chemotherapeutic drugs.

6. Mechanism by ultrasound-mediated microbubble destruction

Ultrasound facilitates the delivery of drugs and genes. In the insonified field, the presence of microbubbles reduces the peak negative pressure which is necessary to enhance drug delivery. This happened because microbubbles acting as nuclei for cavitations decrease the threshold of ultrasound energy. Microbubble gets destroyed by ultrasound due to the gradual diffusion of gas at low acoustic power, formation of a shell defect with diffusion of gas, immediate expulsion of the microbubble shell at high acoustic power, and dispersion of microbubbles into several smaller bubbles.

7. Mechanism by cavitation of the bubbles

It is characterized by rapid destruction of contrast agents due to a hydrodynamic instability during large amplitude oscillations, and is directly dependent on the transmission pressure [43]. Cavitation of the microbubbles increases capillary permeability and delivery of material to the interstitial tissue. When cavitation occurs, this may impart a ballistic effect to drive the drug from the vasculature into or through the vessel wall. Cavitation events will be intimately associated with the drugs themselves (**Figure 4**) [34]. There are two mechanisms for drug delivery in microbubbles that are incorporation of drug and drug release from these microbubbles.

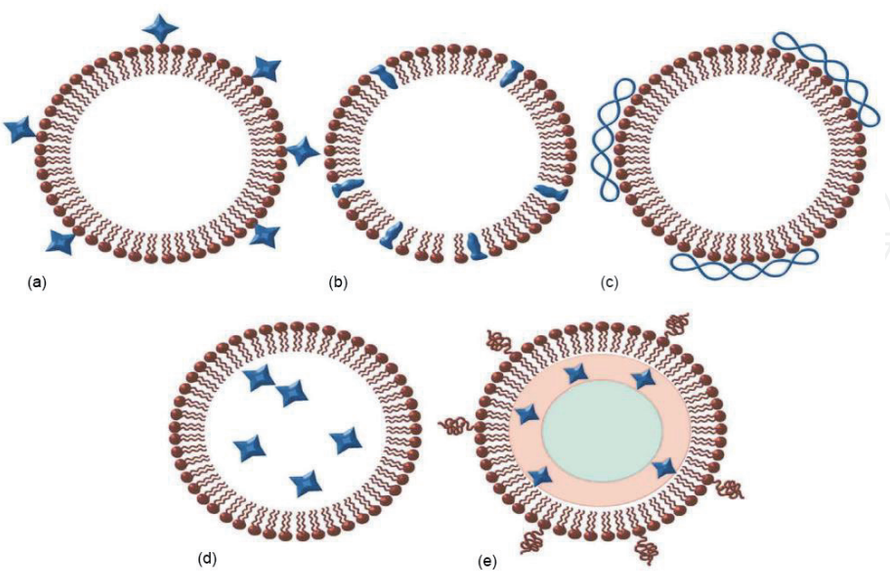

Figure 4.
Different ways microbubbles can transport drugs. Drugs may be attached to the membrane surrounding the microbubble. (a) Drugs may might also be formulated to load the interior with drug and gas, or be imbedded within the membrane itself. (b) Materials, e.g. DNA, may be bound noncovalently to the surface of the microbubbles. (c) Microbubbles hydrophobic drugs can be incorporated into a layer of oily material that forms a film around the microbubble, which is then surrounded by a stabilizing membrane. (e) In this example a targeting ligand is incorporated on the membrane allowing targeted delivery of the drug. Note that although in these examples the stabilizing materials are shown as lipids, but could also be polymeric materials [33].

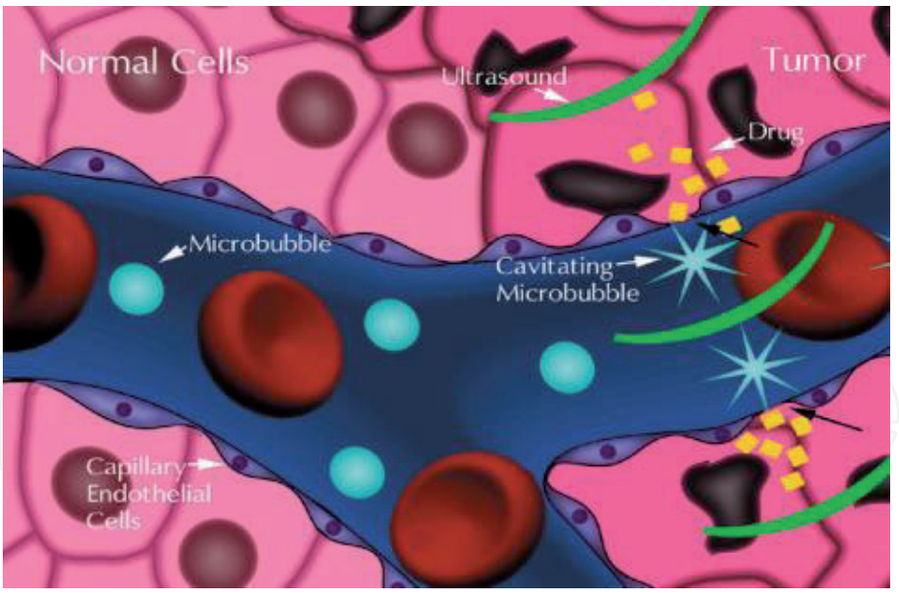

Figure 5.
Drug release from microbubbles by cavitation.

1. **Drug incorporation into microbubbles:** incorporation of drug molecule in the microbubbles in a following way (1) incorporation of drug molecule only within bubble, (2) incorporation of drug molecule within cell membrane, (3) attachment of drug molecule to microbubbles by covalent bonds, (4) attachment of drug molecule to microbubbles by ligand (ex avidin-biotin complex), and (5) incorporation of drug molecule in multiple layer of microbubbles. Microbubbles are able to cross the BBB through above process.

   In **Figure 3** by attaching a targeted ligand such as monoclonal antibody a targeted microbubbles are developed. These are specific for endothelial marker as microbubbles. To assess vascular pathology targeted ultrasound contrast agent are used ex: P-selectin, ICAM-1, GpIIb/IIIa, the αv integrins.

2. **Release of drug from microbubbles:** microbubbles on application of ultrasound undergo a process known as cavitation. Ultrasound causes the microbubble to burst or break. The body fluids begin to insonate on cavitation to create acoustic cavitation. After oscillating microbubbles produces increase small eddy, this increases the permeability of cell membrane and drug passes across the membrane. Microbubbles also release the drug by phagocytosis mechanism. **Figure 5** describe the delivery of drug through fusion mechanism in which the phospholipid microbubble fuse with phospholipid bilayer of cell membrane and releasing of drug or gene into the cytoplasm of cell membrane. By this mechanism the gene get directly transfer to the nucleus of the cell [44–47].

8. Advancements in nano-enabled therapeutics for HIV management

Human immunodeficiency virus (HIV) is a deadly infectious disease worldwide [48–50]. The World Health Organization confirms 0.35 million HIV-infected people. Apart from them, 28 million people are eligible for antiretroviral therapy (ART and only 11.7 million could afford antiretroviral (ARV) drugs. But the ultimate challenge in highly active ART is the elimination of HIV-1 reservoirs from the peripheral nervous system and central nervous system (CNS) [51]. The integration of HIV-1 genome with host genome causes viral latency in the

periphery and in brain. However, the inability of ART to penetrate the blood–brain barrier (BBB) after systemic administration makes brain as one of the most dominant HIV infection reservoirs [48]. Recently, dual therapy, i.e., an optimized cocktail of two ARV drugs, has been introduced to manage HIV infection by Kelly et al. [52]. The authors claimed that the dual therapy containing tenofovir (TEF) exerted more therapeutic advantages than triple therapy. Furthermore, the selection of appropriate drug according to the patient condition is very essential because this therapy may reduce virologic efficacy in HIV-infected patient while lowering CD4 counts per high pre-ART HIV-1 RNA level. This report stated that new nanoformulations (NFs) of LA cabotegravir (CTG) and rilpivirine (RPV) may have bright future aspect for HIV therapeutics. This viable dual therapy is useful to manage ART options and performance, which lowers the costs and the globally unmet needs of pill-fatigued and adherence-challenged individuals [52]. The pharmacologic profile of CTG has great potential for the treatment and prevention of HIV-1 infection. This drug has half-life of 40 days and at a low dose showed therapeutic action, so that monthly and bimonthly oral administration in the form of tablet would be enough to control HIV infection [53]. As the significant advancement made in antiretroviral drug for HIV very few efforts have been developed for effective anti HIV vaccine [54]. To cure neuro HIV the inability of effective anti HIV therapeutic agents to cross the complex integrity of the BBB is the major challenge, so neuro HIV is incurable in the brain. Specific receptor binding, focused ultrasound, microbubble assisted focused ultrasound and magnetic field based approaches have been demonstrated to open the BBB for the delivery of therapeutic agents. Due to bigger in size receptor-functionalized therapeutic cargos affect efficacy whereas an externally stimulated approach results in transient BBB opening, which may also allow the delivery of unwanted agents to the brain [55].

Fluoresce activated cell sorting (FACS) is a standard method for diagnosis of AIDS but having high cost and beneficial only in area where large number of HIV patient resides. The second standard technique is Magnetic activated cell sorting (MACS) used for CD4 cell counting. It involves mixing of sample with magnetic beads which get attached to anti bodies. AIDS can be monitored by using the microbubbles which require no expensive equipment and low cost as compare to above methods. In this technique for separation of CD4 T cell lymphocyte from whole blood cell microbubbles are used. By mixing target specific antibody with microbubbles, the microbubbles get float on surface and provide eminent contact between microbubbles and target cell so that target cell attach to microbubbles while non-targeted cell at the bottom side due to gravity [56].

Ultrasound in presence of microbubbles increases plasmid transfusion efficiency *in-vitro*. Microbubbles form pores upto 100 nm by cavitation mechanism which is having short half life. Loading microbubbles with nucleic acid and /or disease targeting ligand may improve efficiency and specificity. Generation of reversible pore in the plasma membrane due to sonoporation increased plasma membrane permeability to marker compounds. Recent studies state that the effect of low frequency 20 Hz by ultrasound on uptake of fluorescent dye calcein having molecular weight 623 Da and radius 0.6 μm into mouse increase cavitation. This data explains that cavitation occur during insonication influence the membrane permeability. Practical and theoretical experiment on microbubble state that the rapid bubble expansion collapse and subsequent shock wave formation can generate shear forces which disturb cell membrane integrity and increased permeability. Further, the geometry of microbubble collapse is itself influenced by adjacent cell membrane like microjet of the surrounding fluid which in transfection medium contains exogenous nucleic acid may get injected in cell [57].

9. Conclusion

Barriers of HIV/AIDS treatment

- HIV is localized in latent cellular and anatomical reservoirs where the majority of therapeutic agents are unable to completely eradicate the virus for the necessary duration.
- The anatomical reservoirs where the HIV get resides are CNS, the cerebrospinal fluid, the lymphatic system, tests, liver, kidney, lungs, the gut and in the macrophage.
- Microphages act as host for viral genetic recombination where it contribute to the generation of elusive mutant viral genotypes
- The other barriers for current therapeutic drug regimens do not fully eradiate the virus from cellular and anatomical reservoirs. In certain cases patient requires to take daily pills which produce patient adherence. These agents have side effect and in some patient resistance develops.
- Current drug therapy can lower the systemic viral load below the detection limit therefore on discontinuation of treatment, there is relapse of the infection occur from the reservoir sites and a potential for resistance develops.

Microbubbles-based drug delivery for HIV/AIDS treatment

- Microbubbles-based drug delivery systems produce complete eradication of viral load from the reservoir sites.
- Micro bubbles containing antiretroviral drugs bind to the CD4+ T cells and macrophages, and reach to latent reservoir of CNS, the cerebrospinal fluid, the lymphatic system, tests, liver, kidney, lungs, the gut and in the macrophage.
- Microbubble-based drug delivery systems deliver antiretroviral drugs in vitro and in vivo.

Microbubble as therapeutic agents

- Microbubbles have average size less than that of red blood cell so it get penetrated into the small blood capillaries and releasing the drug.
- Microbubbles are used as tool for gene delivery.
- Microbubbles can generate strong signal so lower dose of intravenous required and also used in angiogenesis.

Gene therapy for HIV/AIDS treatment

- Gene therapy remove HIV completely from infected cells, as shown by reductions in the cells' overall rate of HIV production. Gene-editing technique.
- Gene therapy based on siRNA has shown promise for HIV/AIDS treatment. Microbubbles platforms for delivery of siRNA for HIV/AIDS treatment are in their early stages but recent work has been met with optimism.

Immunotherapy for HIV/AIDS

- Microbubbles, loaded with both antigen mRNA as well as immunomodulating Trimix mRNA, which can be used for the ultrasound-triggered transfection of dendritic cell (DC).

- DC sonoporation using microbubbles loaded with a combination of antigen and TriMix mRNA can elicit powerful immune responses *in vivo*, and might serve as a potential tool for further *in vivo* DC vaccination applications.

Microbubble used as preventive HIV/AIDS Vaccine

- New approaches are always being explored for development of an effective HIV/AIDS vaccine.

- For delivering DNA various polymer and lipid- based microbubbles have been used.

- Microbubbles encapsulate antigens in their core and cross link to antigen CD4+ and CD8+ T cells. These absorbing antigen allow B cell to generate responses.

- Microbubble vaccines can be given by different route of administration.

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Author details

Harsha Virsingh Sonaye[1*], Rafik Yakub Shaikh[2] and Chandrashekhar A. Doifode[1]

1 Shri Sachhidanand Shikshan Santh's Taywade College of Pharmacy, Nagpur, India

2 K.E.M. Hospital Research Centre, Pune, India

*Address all correspondence to: harsha_20054@rediffmail.com

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