



A Review On Nanobubbles Unleashed: A Breakthrough In Targeted Cancer Therapeutics

Ms. Hrutika M, Ranade*, Mr Mayur T. Gaikar

PRES'S College of Pharmacy(For women),Chincholi,Nashik

Abstract

Nanobubble technology, characterized by gas-filled bubbles smaller than $1\mu\text{m}$, presents a novel approach for enhancing drug delivery in cancer therapy. These nanobubbles facilitate targeted therapy by improving the biodistribution and bioavailability of therapeutic agents at tumor sites, leveraging their ability to extravasate through leaky vasculature. The structural composition of nanobubbles, primarily consisting of various gases encapsulated by a shell made from proteins, lipids, and polymers, plays a crucial role in their stability and efficacy. This review explores the unique physicochemical properties of nanobubbles, various methods for their production and characterization, and their applications in targeted drug delivery, gene therapy, and theragnostic, and overcoming challenges such as drug resistance and toxicity. The promising results from in vitro and in vivo studies highlight the potential of nanobubbles as a versatile tool in cancer treatment, underscoring the need for further clinical trials to validate their safety and effectiveness.

Keywords: Nanobubble technology, Drug delivery, Cancer treatment, Targeted therapy, Ultrasound, Cell membrane permeability, Enhanced permeability and retention (EPR)effect

Introduction

Nanobubbles are aqueous solution concavities that convey gas and have a size range of less than $1\mu\text{m}$. Round and globular in shape, bubbles have a gas-filled core and a shell that give them noticeable dynamic features.(1,2,3) Nanobubbles are nanometer size bubbles having different constituents of varying physicochemical characteristic for the inner core and outer shell.(4) The shell is mostly made up of proteins, lipids, polymers, surfactants, and polyelectrolyte multilayers, while the core base can be charged with a variety of gases, including air, carbon dioxide, sulfur hexafluoride, perfluorocarbon, and sulfur dioxide.(5)(figure 1) The composition of their shell can have a major effect on a bubble's half-life because it controls the exchange of gas between the shell core and the surrounding substance. Actually, the characteristics that contribute to the overall stability of nanobubbles are shell thickness and elasticity. (6)

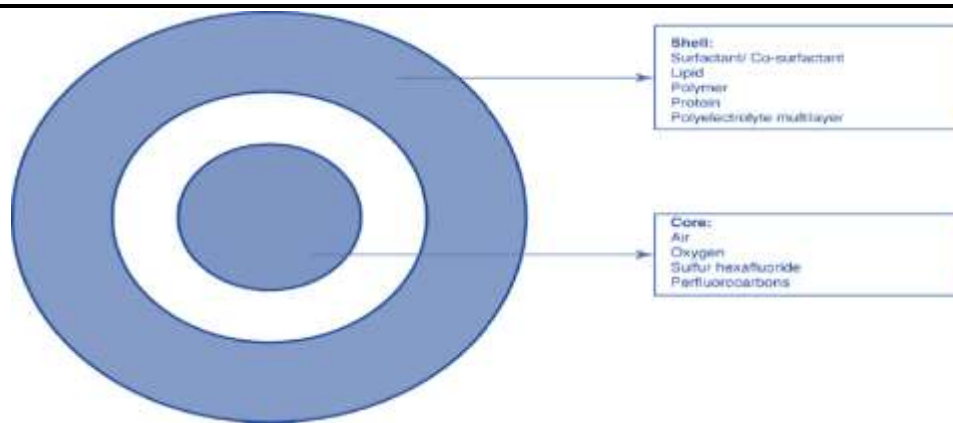


Figure 1-Schematic representation of nanobubble structure.

These sub-micron-sized nanobubbles (NBs) are being developed to increase the drug's biodistribution at the intended diseased location, as well as its stability and bioavailability. Due to their size, microbubbles are too big to be released from the bloodstream.(4) Extravasation of blood vessels into surrounding tissues is facilitated by nanobubbles, which enhances the location and efficiency of delivery.(4,7) However, even in solid tumors, which frequently have leaky vasculature and inadequate lymphatic drainage, MBs are unable to exit the vasculature, which results in extravasation and retention of macromolecules, a phenomenon known as the enhanced permeability and retention, or EPR effect.(8,9)

Cancer is a lethal disease that is characterized by uncontrolled cell growth and spread due to a mutation in the tumor suppressor gene p53. In spite of the progress made in cancer therapy, cancer remains one of the major global health concerns. In addition to the patient's physical health, cancer also has an impact on the patient's emotional and financial well-being as well as that of society. Thus, the development of cancer diagnosis and treatment approaches is necessary. There are several possible therapies for cancer, such as radiation therapy, surgery, and chemotherapy (10) but the major concern associated with available antitumor agents is the non-specificity. They have negative side effects because they damage both malignant and healthy cells. Chemotherapy is greatly impacted by toxicological issues at the tumor site brought on by the non-specific targeting. (10) Solubility of the drugs is the other major concern affecting their therapeutic effectiveness. The majority of anticancer medications in use are hydrophobic and have limited aqueous solubility; as a result, their therapeutic efficacy is reduced during biomembrane extraction. Because of this, creating targeted therapies that target cancer tissues is crucial to achieving the intended therapeutic benefits of antitumor medications [11] with the development of nanotechnology. Also, drug resistance causes more than half of cancer-related deaths.(12) Many factors contribute to tumor cell drug resistance, such as enhanced DNA repair capacity, genetic mutation, heterogeneous biological metabolism, blockage of the apoptosis pathway, microenvironmental alterations, and drug target modifications. Multidrug resistance (MDR) during prolonged chemotherapy is another mechanism.(13) All of the previously discussed processes decrease the effectiveness of drugs and make treating tumors more challenging.

Chemotherapeutic medications, however, are less effective because of negative side effects such systemic toxicity. (14) Through an increase in therapeutic dose at specific areas and a decrease in the plasma levels of circulating medicines, local administration of chemotherapeutic medications may lessen toxicity. The non-invasive and targeted feature of nanobubbles has generated significant interest as a possible drug delivery mechanism. Compared to conventional microbubbles, nanosized particles are more effective at crossing the capillary wall and can reach their destination safely. (15) In this perspective, nanobubbles are widely studied and explored as a potential targeted therapeutic intervention for cancers. This review aims to describe the properties of nanobubble systems and their applications in medicine, particularly in the treatment of cancer.

History of nanobubble technology

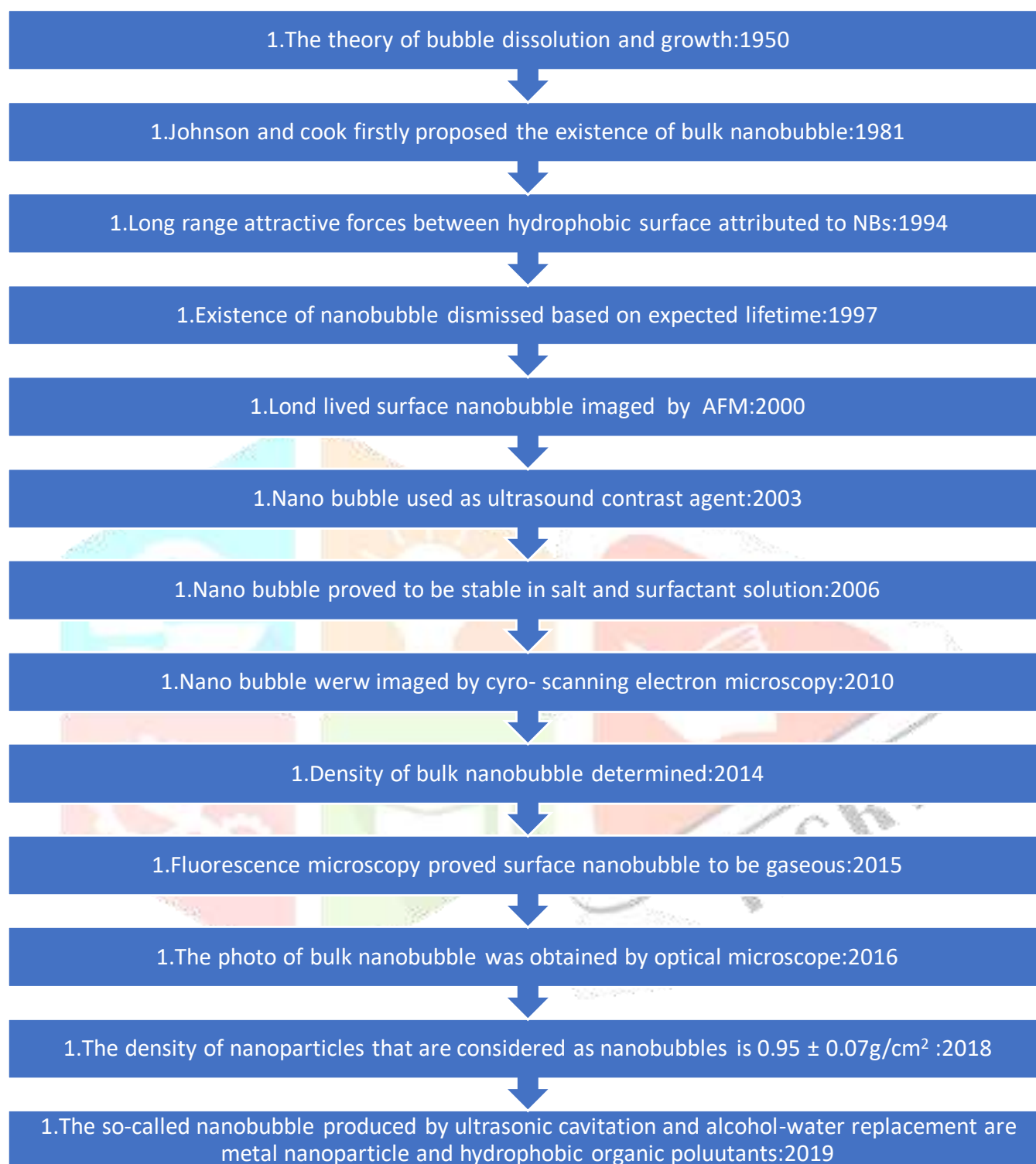


Figure 2: History of nanobubble technology(16,17,18)

Nanobubble characteristics

An optimal size for a nanobubble is less than 1 μm . According to the International Organization for Standardization (ISO), a nanobubble is a gaseous domain inside a medium. (19) The stability and extended lifespan of nanobubbles have made them useful in a wide range of industries. Some of these industries include food and beverage manufacturing, home chemicals, rust removal, waste management, hydroponics, fossil fuels, and medical products.(20-26) Bubbles that are adhered to a submerged surface or entrapped in a fracture are known as surface nanobubbles, or SNBs for short. The bulk liquid has bulk nanobubbles (BNBs) scattered throughout it. Parker and colleagues originally reported the occurrence of nanobubbles in the early 1990s. They carried out tests to look into the interactions between two hydrophobic surfaces submerged in water. (27) Up to 100 nm-sized SNBs can be seen with the use of the atomic force microscopy (AFM) apparatus.(28)

Several methods, including solvent exchange, gas purging, and oversaturation, can be used to produce nanobubbles. The mechanical processes of agitation, vortexing, sonication, compression, decompression, hydrodynamic cavitation, and microfluidics are used to create BNBs. Generally, aeration is accomplished through mechanical means, like micro/nanobubble generators. Usually, these techniques cause the system to expand even more before buoyancy causes it to escape. On the contrary, the remaining mechanisms cause the system to contract and collapse, which eventually causes nanobubbles to form. (29)

Dynamic light scattering and nanoparticle tracking analysis are the main techniques used to characterize BNBs. Both methods measure the sizes of nanoparticles and rely on light scattering. The resolution is approximately 1 nm, and it is unaltered by the Abbe limit, which is the diffraction limit of a microscope. (30)

Construction of nano bubble in biomedicine

Pharmacokinetics, absorption mechanism, distribution, and clearance in the human body are all influenced by narrow particle size ranges and their low size distributions. (31) Therefore, extravasation and safe transit across blood capillaries and the blood-brain barrier (BBB) are made possible by the small size of nanobubbles.(32) It is possible to cover the surface of the nanobubble and fill the center with different substances. The shell maintains the elasticity, stability, half-life, and protection of the bubble core by reducing the rate of gas diffusion and preventing disproportionation, coalescence, and excretion. (33) The total stability of the core is improved by the in situ integration of numerous gases, each of which facilitates the binding of another. Because the gas inside the core functions as a harmonic oscillator, ultrasonication is frequently utilized to transport nanobubbles. The intensity of the ultrasonication determines the reactivity of the bubbles. (Figure 3) It has been shown that high-intensity ultrasound causes the nanobubbles to collapse, whereas low-intensity ultrasound may lead the bubbles to a desired location. Exogenous substances can enter the cells or tissue as a result of the localized increase in membrane permeability caused by this collapse. A number of mechanisms, including cavitation, sonoporation, acoustic radiation force, acoustic streaming, and ultimately a thermal effect, are triggered when ultrasonic waves are directed towards nanobubbles. The acoustic intensity, which can be categorized as low or high, determines the oscillation intensity. High intensity oscillations move in a non-linear, asymmetric way with rapid expansion and bubble collapse, while low intensity oscillations are characterized by consistent movement. Sonoporation is the process via which the oscillation of the bubbles creates micropores in the cell membrane. (34)

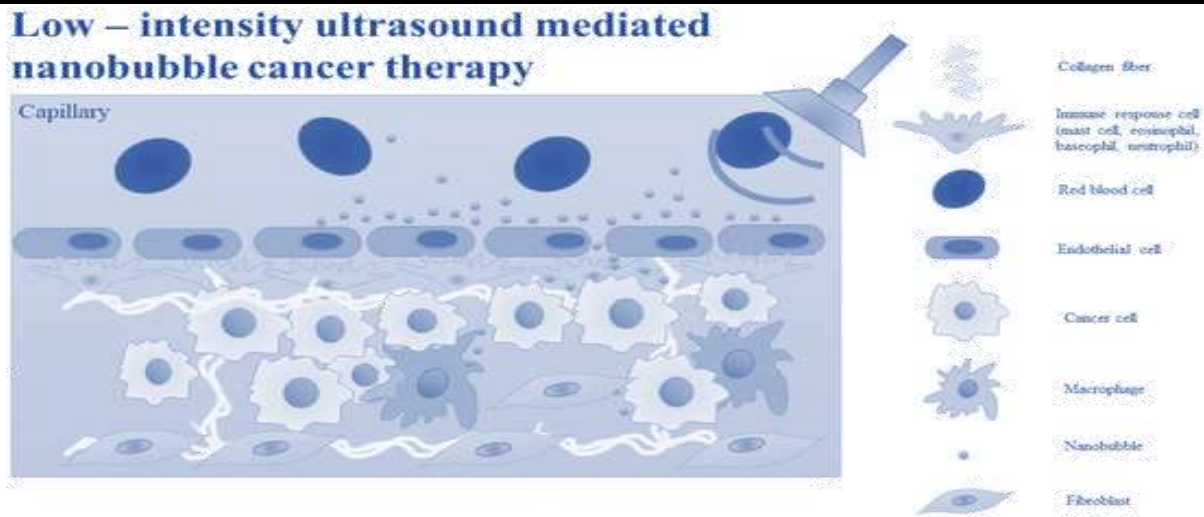


FIGURE 3: Low-intensity ultrasound-mediated nanobubble cancer therapy. (35)

Composition of nanobubble

There are two primary types of nanobubble components—the inner core and the outside shell—each with unique physicochemical properties. (Figure 1) While sulfur hexafluoride and perfluorocarbons are present in the core air, surfactants, polymers, and proteins make up the majority of the shell. Polymers, surfactants, phospholipids, lipids, and proteins can all be used to create the hard or soft shells of nanobubbles. (36)The stiffness of the bubbles, their resistance to rupture in the ultrasonic pressure field, and the ease with which the reticuloendothelial system recognizes and clears them are all influenced by the composition of the shell. The shell serves as a barrier between the encapsulated gas and the surrounding aqueous medium, limiting gas dissipation, while the core is a single low-density chamber that makes up the majority of a particle's volume. A few common examples of core and shell are represented in (Table 1)

TABLE 1: Few common examples of core and shell material of nanobubble (36)

Core	Shell
Air	Surfactant/cosurfactant
Oxygen	Lipid
Sulfur hexafluoride	Polymer
Perfluorocarbon	Protein
Octofluoropropane	Polyelectrolyte multilayer

Characterization of Nanobubble

Typical characterization is usually carried out for the NBs is appended below

TABLE 2: Typical parameters for nanobubble characterization (37)

Parameter	Brief Detail
Bubble size	dynamic light scattering using photon correlation spectroscopy (PCS) at 25 °C
Zeta potential	Zeta Plus Analyzer, Surface potential Cytometry
Magnetic properties of magnetic nanobubble	Measured by using vibrating sample magnetometer (VSM)
Structure of nanobubble	Scanning electron microscopy (SEM) transmission electron microscopy (TEM)
Dissolved oxygen	Electrochemical sensing, fiber optic-based sensing, and fluorescence quenching
Bubble concentrations	hemacytometer

Systems based on nanobubbles for cancer treatment

1 Plasmonic nanobubble therapy

People have been suffering from cancer for decades. One of the greatest treatment choices for removing solid tumors is surgery. There is a chance that the cancer may return, nevertheless, as surgery by itself cannot guarantee that the cancerous mass will be completely removed. In spite of this, PDT and PTT have demonstrated encouraging outcomes in the treatment of cancer; yet, their drawbacks include the possibility that healthy cells may also be impacted in addition to malignant cells (38), emphasizing the need for targeted therapy. The use of nanobubble treatment (NBT) is essential for proving targeted cancer cell destruction without endangering healthy cells. The nanoparticles were able to be expelled from the body because they broke apart and became smaller (less than 10 nm) when exposed to laser light. (39) Recently, NBT has been employed by numerous researchers to treat cancer in vitro. Juan et al. showed how NBT for endosomal escape and gene silencing could deliver siRNA or medications without interfering with cell homeostasis. The formation of vapor nanobubbles upon laser irradiation of gold nanoparticles (AuNPs) resulted in endosomal membrane rupture, while heat diffusion produced endosomal membrane permeabilization. The release of small interfering RNA (siRNA) proved effective in the mechanisms of heat generation and nanobubble creation. Whole transcriptome analysis studies on cell homeostasis revealed improved cell recovery in 24 to 48 hours. (39) In order to cause immunogenic cell death in breast cancer cell lines, Hieu et al. employed NBT. Gold nanorods (AuNRs) exposed to a single laser pulse produced micron-sized bumps in the cytoplasm of breast cancer cells. Heat shock protein 70 (Hsp70), adenosine triphosphate, and human high mobility group box 1 (HMGB1) are examples of chaperon proteins whose expression was increased by damage-associated molecular patterns (DAMPs). Consequently, the expression of DAMPs caused the dendritic cells to become activated.(40)

The selective transfection of genes through the use of plasmonic nanobubbles, a novel cellular agent having a dual and adjustable mechanical and optical activity. Through the process of plasmon resonance, vapor nanobubbles are created around plasmonic nanoparticles by temporarily heating them with a brief laser pulse. This results in a localized mechanical effect that is non-thermal on the cell. Gene therapy can be safely and effectively carried out with plasmonic nanobubbles, which can deliver targeted genes at the single cell level in a single pulse process. This strategy is presented here to round out the possible nano-scale systems involved in gene transfer, even though it has nothing to do with a nanobubble formulation.(41) Demonstration of plasmonic nanobubble therapy mechanism on cancer cell shown in figure 4

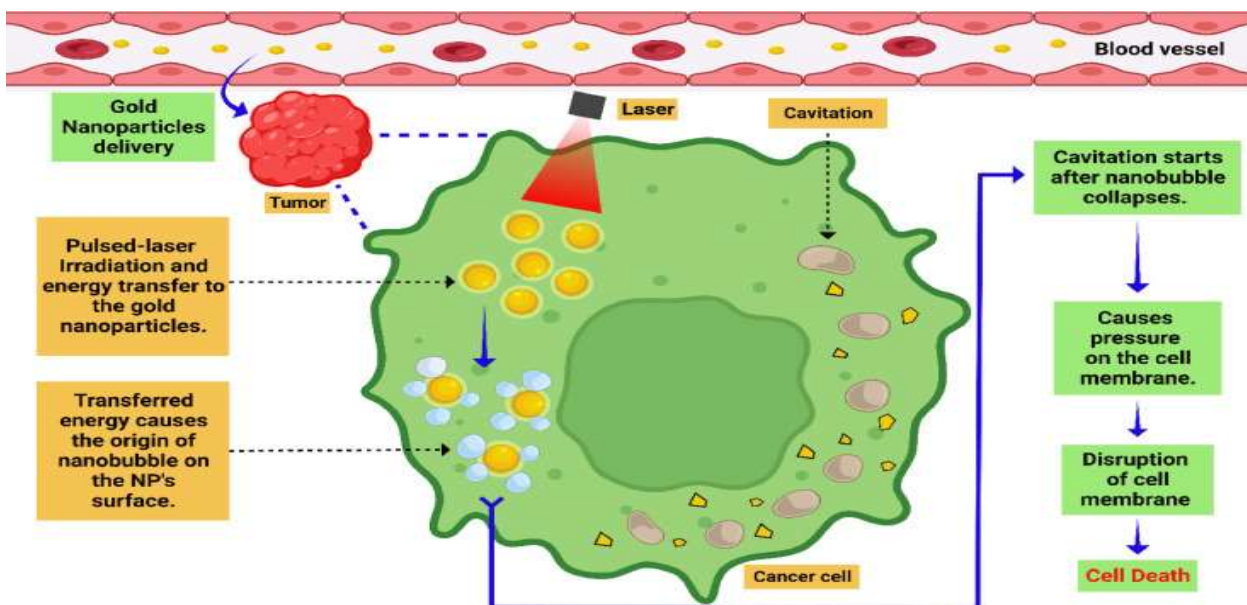


Figure 4: Demonstration of plasmonic nanobubble therapy mechanism. (42)

2 Nanoparticles used for nanobubble generation

Multiple features can be displayed by a single type of nanoparticle. For example, AuNPs/AuNRs demonstrated PDT/PTT effects (Fig. 5) at low laser fluences, but nanobubble effects at high laser fluences.(43) A necessary component of the nanobubble effect is SPR. Free electrons in the outermost orbital oscillate due to a phenomenon called surface tension resonance (SPR), which absorbs and transfers energy to the surrounding (cytoplasm) to create nanobubbles.(44) Plasmonic nanoparticles, which demonstrate surface plasmon resonance (SPR) properties, include semiconductor nanoparticles and gold (Au), silver (Ag), and platinum (Pt). The size of the particles (1–100 nm) is a distinguishing feature of nanoparticles. Chemical and biological alterations are equally significant as particle size. Accordingly, altering particles enhances their stability, cellular uptake, biocompatibility, and energy absorption capability. In order to cure cancer in vitro, Juan et al., Hieu et al., Eline et al., and Felix et al. changed the surfaces of AuNPs/AuNRs using hyaluronic acid, (11-Mercaptoundecyl)-N, N, N-trimethylammonium bromide (Mutab), and polyethylene glycol (PEG).(43,45,46)

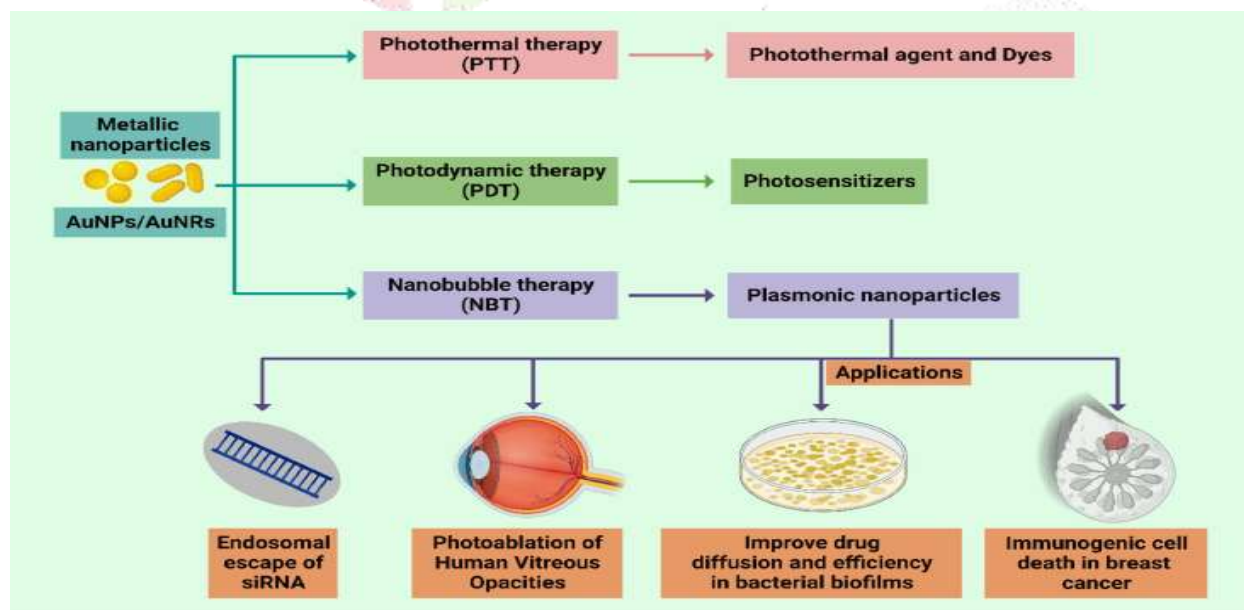


Figure 5: Particle involved in nanobubble therapy and its application

3 Ultrasound-based nanobubble system in cancer theragnostic

Research on ultrasound-assisted nanobubble (US) generation as a substitute for pulsed laser-induced nanobubble generation has been quite extensive. Real-time imaging is made possible by ultrasound (US)-assisted nanobubbles (NBs), and sonoporation caused by ultrasound has the ability to temporarily puncture or open cell membranes. (47,48) Thus, combining the applications of ultrasound with NBs has recently demonstrated increased localization of anticancer molecules in tumor tissues with triggered release behavior.(49)

Sonoporation, the biophysical process of cell penetration by ultrasound in conjunction with NBs, is the foundation of ultrasonic delivery technology. In contrast to other nanoparticles, NBs possess a unique characteristic that causes them to "collapse" when ultrasonic energy is applied, which causes NBs to implode and alters the permeability of the cell membrane.(50) Ultrasonic cavitation is the result of sufficiently high ultrasonic energy; bubbles, or cavitation nuclei, in the liquid vibrate and expand, continuously absorbing energy from the acoustic field and collapsing until the energy exceeds a threshold. Ultrasonic cavitation, which is brought on by ultrasonic radiation, results in gaps in the cell membrane that have a diameter of approximately 300 nm. Stable cavitation is defined by recurring, non-collapsing oscillations of nanobubbles, which cause local flow and shear stress on adjacent cells, thereby increasing blood vessel permeability.(51) Recently, specific nanocarriers have gained enormous popularity as an important means of drug and gene distribution owing to their properties. Further, they are capable of attaining the maximum concentration of cytotoxic agents in a site-specific manner. Antitumor drug delivery by nanocarriers is being targeted using a variety of targeting techniques. One particularly successful method of targeting the delivery of anticancer drugs is the use of various antibody-conjugated nanoparticles (NBs) that bind to ligands in cancer cells and tissues. NBs may be combined with Affibody® as a directed probe in the manufacture of nano-sized ultrasonic contrast agents.(52,53)

A key factor in the effectiveness of NBs is biological changes in tumor tissue. Normal tissue has a thick microvascular endothelial gap and intact endothelial cell structure; in contrast, neovascular endothelial holes in solid tumor tissues range in size from 380 to 780 nm, and endothelial cell structural integrity is compromised. As demonstrated in Fig. 5A, molecules or particles of particular sizes thus have a tendency to accumulate more in tumor tissues than in normal tissue. (54)The enhanced permeability and retention (EPR) effect is a phenomenon that seems to be the mechanism via which tumor tissues undergo passive targeted treatment.(55) Preclinical studies have demonstrated a notable advancement in anticancer efficacy as compared to standard chemotherapy for medications or gene delivery-targeted systems based on EPR.(56)

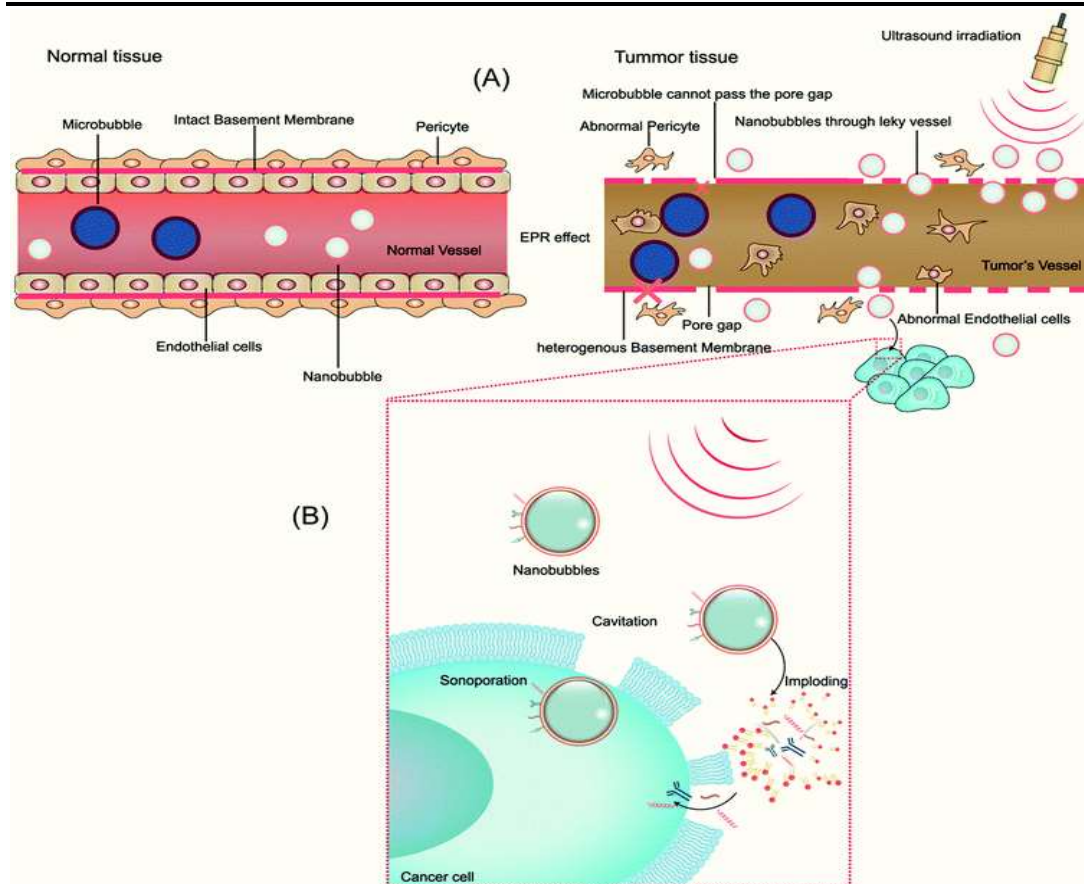


Figure 5: Schematic representation showing the effect and interactions of biological factors on ultrasound combined with nanobubbles and description of numerous mechanisms via NBs for the transport of drugs/genes to tumor tissues. (A) Mechanism of EPR effect in tumor and normal tissues, where microbubbles cannot pass through the endothelial pore but nanobubbles can in tumor tissues. (B) The biological impact of using ultrasonic in conjunction with NBs and enhanced cancer cell membrane permeability, which causes NBs to implode at the same time.(49)

3.1 Ultrasound combined with NBs for the delivery of anticancer drugs

It was originally proposed that drug-associated NBs be synthesized and coupled with US for cancer chemotherapy. According to Suzuki et al. (57), permeabilizing four types of tumor cells and boosting the effectiveness of anticancer medications were achieved with the use of NBs in combination with US. It was successfully possible to increase the cell susceptibility to cisplatin and 5-FU by using NBs and US. To improve the localized efficacy and targeting of oxygen-trapping NBs for the reversal of hypoxia in non-muscle-invasive bladder cancer (NMIBC) tumors, US may be utilized for directing this NBs. The capacity to precisely guide non-invasive and steady ultrasonic imaging navigation-based NBs carrying a payload offers enormous promise for controlled cargo distribution engineering and hypoxia reprogramming for a wide range of therapeutic applications.(50) Using US in combination with NBs loaded with apatinib and targeted with the liver cancer homing peptide (GPC3) may be a novel approach for treatment of liver cancer .(58) NBs containing doxorubicin (DOX) showed exceptional encapsulation capabilities for chemotherapeutic agents and the ability to kill cancer cells when exposed to ultraviolet light. The delivery of DOX into tumor cells in vitro was accomplished by US irradiation in conjunction with DOX-loaded NBs. Furthermore, in mammals, NBs containing DOX effectively dispersed DOX to tumor cells. In order to distribute DOX in an ultrasonic-sensitive manner, chitosan nanoparticles (NBs) are an effective and non-invasive drug delivery medium.(59) Innovative nanocarriers called perfluorocarbon NBs have the ability to evaporate upon contact and change into micron-sized gas bubbles via the use of heat shock. When PFC nanodroplet vaporization occurs, the micron-sized gas bubbles will react strongly with ultrasonic irradiation. The bubbles can be used for tumor tissue imaging and therapy.

Rapoport et al.(60) encapsulated liquid perfluoropentane (PFP) containing doxorubicin using polymeric micelles. By raising the temperature to 37 °C in this apparatus, the PFP nanodrops were able to evaporate and transform into larger bubbles. This suggested that the US exposed to tumor radiation could cause nanobubbles

to enlarge into larger bubbles many times larger than droplet size through the process of vaporized nanodrop coalescence and thermodynamic effects following phase transition. They demonstrated how polymer-stabilized PFP NBs can transmit doxorubicin to tumors that have been xenotransplanted and showed how this approach can restrict tumor growth. In another study, PFP NB copolymers stabilized the amphiphilic chain to facilitate paclitaxel delivery. Both in vivo and in vitro results with tumor cells were intriguing. When systemic administration PFP nano-emulsions containing paclitaxel were used in conjunction with 1 MHz US, positive tumor regression was observed in the breast, ovaries, and pancreas.(61) Studies by Rapoport et al. revealed that the therapeutic chemicals put inside the bubbles are released and their intracellular absorption is enhanced by the movement of the bubbles from droplet to bubble and by their oscillation. Based on the droplet to bubble transition (Fig. 6), the suggested method for US-mediated drug transfer by polymer-linked perfluorocarbon NBs. This is meant to help with the capsulated pharmaceutical distribution process, in which the medication is "ripped off" from the droplet's surface due to US activity.

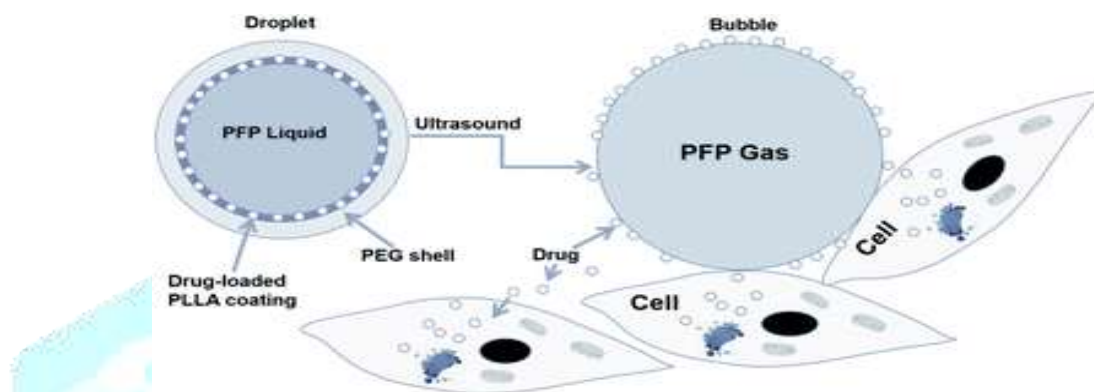


Figure 6: A diagram showing how ultrasound is used to transport a medication from a nanodroplet into a cell.

NBs can be utilized in drug delivery complexes with liposomes directed by US (Fig. 7). Drug delivery using US-targeted liposome complexes may also utilize the use of 88 NBs. Effectively designed nanobubble-paclitaxel liposome complexes (NB-PTXLp) facilitated drug delivery to tumor cells stimulated by ultraviolet light. Liposome binding to the NB surface had no effect on the NB-PTXLp theragnostic device's imaging capabilities. Additionally, the cavitation of NBs brought on by ultrasound irradiation results in tumor cell sonoporation, which raises the permeability of the cells. This resulted in the greatest anticancer potency of NB-PTXLps in comparison to the generic Abraxane and Paclitax formulations and enabled cellular absorption of PTX-loaded liposomes. (62)

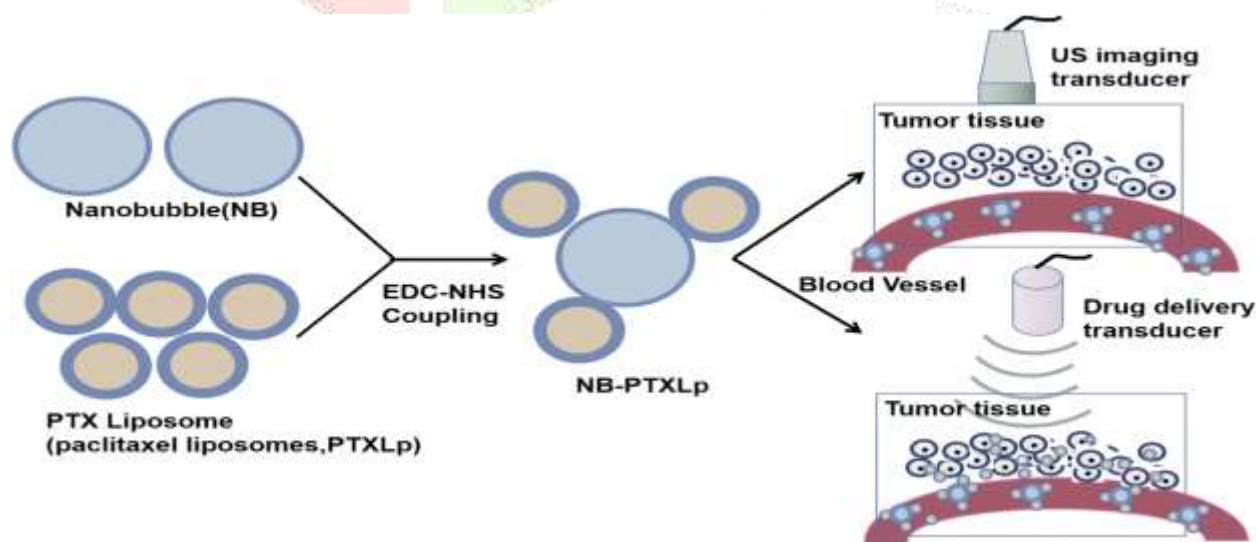


Figure 7: Effectively created, nanobubble-paclitaxel liposome (NB-PTXLp) complexes enhanced drug delivery in tumor cells stimulated by US irradiation.

Nanobubble Modifications against Cancer

The medical industry has profited recently from the versatile applications of nanobubble technology as ultrasonic contrast agents and therapy tools, despite the fact that the long-term presence of nanobubbles (which can range from weeks to months) is still not fully understood [63]. These include theranostics agents, targeted gene delivery [64], skin disease treatment [65], oxygen delivery to hypoxic tumors [66], ultrasound-triggered drug administration and imaging [67], and neurological disorders. The ability of nanobubbles to penetrate areas that other therapies find challenging to reach sets them apart from microbubbles. Size, maintaining stability, and the surface area to volume ratio (S/V ratio) are the primary causes of this. Modifications of nanobubbles against cancer are shown in figure 8

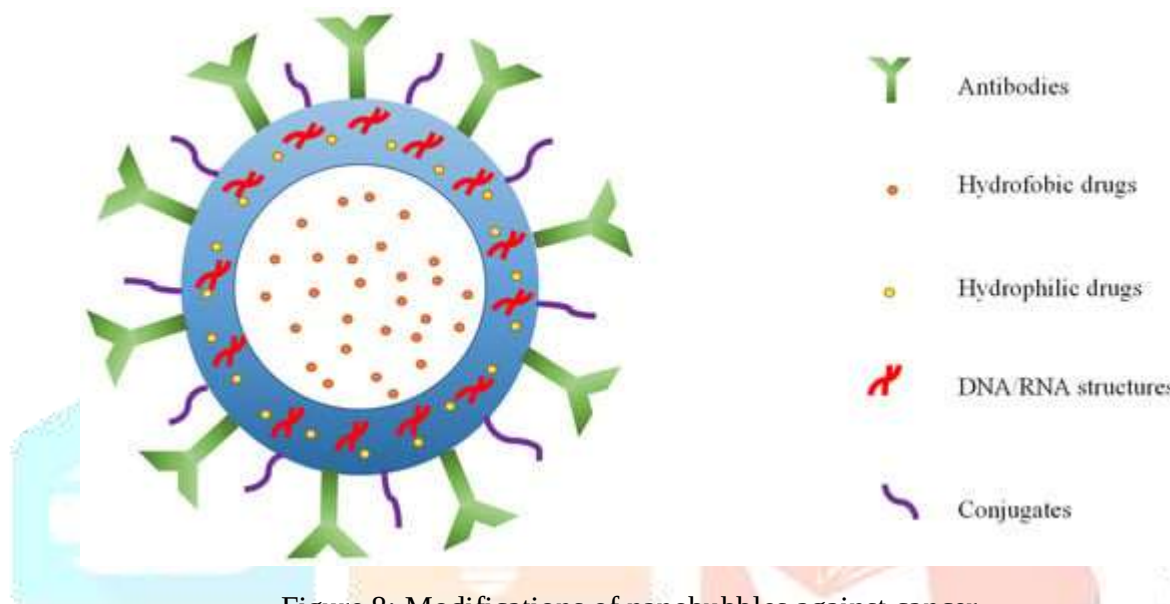


Figure 8: Modifications of nanobubbles against cancer

Doxorubicin belongs to the class of drugs known as anthracyclines and anti-tumor antibiotics [68]. It functions partly by interfering with DNA's capability to function. Doxorubicin suppresses the synthesis of macromolecules and intercalates DNA in two different ways. It stops topoisomerase II, an enzyme that opens DNA supercoils to permit transcription, from developing. Once the DNA chain has been broken for replication, doxorubicin stabilizes the topoisomerase II complex, blocking the release of the DNA double helix and so stopping the replication process. Furthermore, it can increase the production of free radicals of the quinone type, increasing its cytotoxicity [69]. The polysaccharide chitosan has received significant interest because of its natural source, biodegradability, biocompatibility, antibacterial properties, and minimal immunogenicity. One of the most prevalent biological substances found in nature is chitosan, which is a derivative of N-deacetylated chitin [70]. In the Zhou et al. study [59], doxorubicin was incorporated into chitosan nanobubbles and administered to breast cancer cells in vitro using ultrasound. The proportion of apoptotic cells was much higher in the group treated with doxorubicin hydrochloride and chitosan nanobubbles containing a perfluoropropane core when paired with ultrasound than in the group treated with free DOX. The outcomes of this study and others mentioned below are shown in Table 3

Tumors in low-oxygen environments are more resistant to chemotherapy, radiotherapy, and photodynamic therapy [72]. Tumor resistance to these therapies has been connected to a protein called HIF-1 α , which is stabilized under hypoxic conditions [73]. Tumor cells that express many genes related to angiogenesis, apoptosis, pH control, and cellular differentiation transition to anaerobic metabolism due to HIF-1 α . Many studies are looking for ways to inhibit or deactivate HIF-1 α in order to improve the efficacy of treatments. The effects of doxorubicin hydrochloride-loaded oxygen nanobubbles on MDA-MB-231 breast cancer cells and HeLa pelvic cancer cells were investigated in vitro by Khan et al. [74]. The investigation showed that oxygen nanobubbles inhibit HIF-1 α activity in tumor cells and reverse hypoxia.

Paclitaxel is among the several cytoskeletal medications that target tubulin [75]. In contrast to other tubulin-targeting medications that inhibit microtubule assembly, like colchicine, paclitaxel stabilizes the microtubule polymer and shields it from disassembly, impairing mitotic spindle assembly, chromosome segregation, and cell

division in treated cells [76]. Chromosomes are consequently unable to form a metaphase spindle structure. This process stops the progression of mitosis, and prolonged activation of the mitotic checkpoint results in apoptosis or cell cycle return to the G₀-phase in the absence of cell division.[77] Human regulatory peptides known as gastrin-releasing peptides (GRP) trigger the release of gastrin and control the secretion of stomach acid and enteric motor function [78]. The release of GRP by the vagus nerve postganglionic fibers innervating the stomach's bombesin/GRP neurons causes the G cells to release gastrin. The most widely used, very accurate, and successful biomarker for small cell lung cancer (SCLC) diagnosis and treatment is ProGRP [79]. As a result, one potential therapeutic approach for SCLC growth inhibition is to target ProGRP. The effectiveness of a pro-gastrin-releasing peptide-laden nanobubble loaded with paclitaxel against small cell lung cancer was examined by Wang et al. [80] both in vitro and in vivo. Targeting nanobubbles with paclitaxel effectively prevented SCLC cells from proliferating, migrating, and infiltrating while causing these cells to undergo apoptosis.

Table 3. In vitro/in vivo results of nanobubble modifications against different cancers.

Drug/Oxygen/Antibody Delivery Technique	Analyzed Material	Measurement Techniques	Outcomes	References
Doxorubicin hydrochloride and chitosan nanobubbles with a perfluoropropane core in combination with ultrasound (DOX-NBs + US)	MCF-7 cells (Michigan Cancer Foundation-7 cells), breast cancer cells, in vitro	Confocal images with Annexin V staining, flow cytometry	increased percentage of apoptotic cells ($45.7 \pm 1.1\%$ vs. $4.4 \pm 0.9\%$, $p < 0.01$) in comparison to a group that received free DOX	[59]
Doxorubicin hydrochloride-loaded oxygen core nanobubbles with phospholipid shell	MDA-MB-231 (MD Anderson-Metastatic Breast-231 cancer cells), HeLa cervical cancer cells, in vitro	Reactive oxygen species assays (ROS), confocal images, fluorescence, DAPI staining	- Reversed hypoxia - Increased generation of ROS - Suppression of hypoxia-inducible factor-1 alpha activity in tumor cells - Increased drug effects	[74]
Paclitaxel-loaded nanobubbles with anti-pro-gastrin-releasing peptide antibody	SCLC (small cell lung cancer), H446 lung cancer cells, in vitro, in vivo	Reverse-transcription polymerase chain reaction (RT-PCR), Western blot, immunohistochemical detection, CCK-8 assay, flow cytometry, cell scratch test,	- inhibited SCLC cell proliferation, migration, and promotion of cell apoptosis ($28.28 \pm 4.2\%$) - Decreased expression of Bcl-2, surviving, CDK2, MMP-2 ($p < 0.01$),	[80]

		tumor-burden nude mice models	increased expression of caspase-3 and Rb ($p < 0.05$) • Lower tumor weight than the control group (p = 0.001)	
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Future Perspectives in Cancer Treatment

Recent years have seen tremendous advancements in the scientific research of nanobubbles, especially in relation to anticancer treatment. Nanobubble efficacy and good blood capillary perfusion are confirmed by in vivo and in vitro investigations on cancer; however, further clinical trials are required to establish safety and negative effects. Conjugate nanobubbles have been shown in numerous trials to be a more potent therapeutic tool than traditional techniques. However, according to the clinicaltrials.gov website, there aren't any clinical trials with cancer patients going on right now. Research using human subjects address the diagnosis of malaria, hypoxia, pulmonary fibrosis, or amyotrophic lateral sclerosis (ALS). Interestingly, nanobubbles not only function as a tracking tool for cancer immunotherapy but also offer opportunities for preclinical research on therapeutic improvements. Jiang et al. [81] investigated the use of ultrasonic imaging for in vivo post-infusion monitoring of natural killer (NK) cells. NK cell-specific contrast agents, known as nanobubbles, were used in both monotherapy and synergistic treatment with cytokines. Following co-administration of interleukin-2 (IL)-2, the team saw that 3 hours after infusion, NK cells had arrived at the tumor site. The trafficking, infiltration, durability, and anti-tumor activities of these infused immune cells in the tumor microenvironment (TME) offer crucial information for assessing the therapeutic effects following treatment and developing adjuvant techniques to optimize those effects. In general, nanobubbles are the newest area of nanotechnology and have potential applications in cancer treatment since they complement other nanotechnologies (such as nanogels, nanotubes, and nanoparticles) and act in concert with traditional therapy [82].

Conclusion

Nanobubble technology represents a significant advancement in the field of drug delivery and cancer treatment, offering a unique and effective means to enhance therapeutic efficacy while minimizing side effects. Their small size facilitates extravasation through the leaky vasculature found in tumors, improving drug biodistribution and bioavailability. The physicochemical properties of nanobubbles, influenced by their composition and stability, play a crucial role in their ability to navigate biological barrier and deliver therapeutic agents directly on targeted sites. Research highlights the versatile applications of nanobubbles, including their roles in enhancing the permeability of cell membranes, promoting drug release, and overcoming challenges related to drug resistance. Additionally, their compatibility with ultrasound technology further amplifies their therapeutic potential allowing for non-invasive monitoring and precise targeting treatment. Despite the promising results from in vitro and preclinical studies, clinical applications of nanobubble technology are still in their infancy, necessitating further investigation. Future research should focus on optimizing the composition and functionalization of nanobubbles for specific cancer types, as well as conducting comprehensive clinical trials to assess their safety and efficacy in human subjects. efforts between researchers, clinicians and industry stakeholders will crucial to translating these Innovative findings into standard cancer care. Ultimately, the integration of nanobubble technology into cancer therapy could revolutionize treatment protocols, offering new hope for patients and enhancing the overall effectiveness of therapeutic agents. As we continue to explore and refine this technology, it holds the potential not only to improve cancer outcomes but

also to pave the way for advancements in other applications. The future of nanobubble technology is bright, promising a new frontier in precision medicine and targeted therapeutic interventions.

Abbreviations

AFM	atomic force microscopy.
ALS	amyotrophic lateral sclerosis.
BBB	blood-brain barrier.
BNBs	bulk nanobubbles
DOX	doxorubicin.
EPR	enhanced permeability and retention
GRP	gastrin-releasing peptide.
HIF1 α	hypoxia-inducible factor.
MBs	Microbubbles
MDA-MB-231	breast cancer cell line.
NB-PTXLp	nanobubble-paclitaxel liposome
NBs	sub-micron-sized nanobubbles
NBT	nanobubble treatment
NK cell	natural killer cells
PDT	photodynamic therapy.
ProGRP	pro-gastrin-releasing peptide.
PTT	photothermal therapy
ROS	reactive oxygen species.
SCLC	small cell lung cancer
SNBs	surface nanobubbles
US	ultrasound

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