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A review of ultrasound-mediated microbubbles technology for cancer therapy: a vehicle for chemotherapeutic drug delivery

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Literature Review

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Abstract

Background: The unique behaviour of microbubbles under ultrasound acoustic pressure makes them useful agents for drug and gene delivery. Several studies have demonstrated the potential application of microbubbles as a non-invasive, safe and effective technique for targeted delivery of drugs and genes. The drugs can be incorporated into the microbubbles in several different approaches and then carried to the site of interest where it can be released by destruction of the microbubbles using ultrasound to achieve the required therapeutic effect.
Methods: The objective of this article is to report on a review of the recent advances of ultrasound-mediated microbubbles as a vehicle for delivering drugs and genes and its potential application for the treatment of cancer.
Conclusion: Ultrasound-mediated microbubble technology has the potential to significantly improve chemotherapy drug delivery to treatment sites with minimal side effects. Moreover, the technology can induce temporary and reversible changes in the permeability of cells and vessels, thereby allowing for drug delivery in a spatially localised region which can improve the efficiency of drugs with poor bioavailability due to their poor absorption, rapid metabolism and rapid systemic elimination.

Introduction

Cancers encompass a wide variety of diseases that share a common characteristic of unregulated cell growth. According to the Canadian Cancer statistics, an estimated 206,200 new cases of cancer and 80,800 deaths from cancer occurred in Canada in 2017.[1] The traditional therapeutic modalities for cancers are mainly surgery, chemotherapy and radiation therapy; and more recently immunotherapy, hormone therapy and targeted therapy have become commonplace for some cancer treatments. In the past few years, there have been growing investigations towards personalised and targeted treatment options for cancer patients including gene therapy,[2] use of quantum physics principles to understand and treat cancer,[3] use of functionalised gold nanoparticles for cancer therapy,[4] the use of ultrasound-mediated microbubbles for drug delivery[5–67] and combining imaging genomics and radiation genomics.[68] The ultrasound-mediated microbubble technique used as a non-invasive delivery of therapeutic agents for cancer treatment has recently evolved as one of the advanced therapeutic methods for personalised and targeted cancer treatment.[5] In the 1950s, Fellinger and Schmid were the first to use ultrasound as a therapeutic tool. Using this therapeutic tool they were able to successfully treat polyarthritis with ultrasound to increase the permeation of ointment into the inflamed tissue.[10,69]

An ultrasound system uses sound waves to produce images of the internal organs of the body and is usually used to diagnose the causes of pain, swelling and infection in the body's internal organs; to examine babies in utero; to help guide biopsies; to diagnose heart conditions and to assess damage after a heart attack. Ultrasound is a non-ionising diagnostic tool that is safe, cost-effective and non-invasive and the acoustic waves can penetrate several centimetres (up to 15 cm) deep into tissue depending on its frequency and the tissue's density, without significant signal attenuation and distortion. Furthermore, their acoustic energy can be deposited in a small focal region (1–10 mm), to cause various therapeutic effects, such as thermal tissue coagulation[11] and kidney stone comminution.[12]

The application of microbubbles as contrast agents for ultrasound utilisation was originally developed to improve ultrasound signal and to enhance delineation of tissue borders and characterisations.[70] This application is based on a unique principle of the generation of spherical microbubbles with rheological properties that are optimal enough to survive the internal body conditions. The first description of microbubbles as ultrasound contrast agents was in 1968 when Gramiak and Shah studied the contrast phenomena that they observed in the aorta during

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cardiac catheterisation following injection of saline solution and subsequently enhanced backscattered signal generated by microbubbles produced at the time of injection.[71,72] Although the clinical applications of micro-bubbles as drug delivery wa s largely a theoretical concept about two decades ago, several studies[16–67] are currently ongoing and some have achieved promising results, suggesting that most of these therapeutic options have the potential to be used clinically within the next few years. This narrative review paper reports on recent studies on ultrasound-mediated microbubbles as a vehicle for drug delivery and the potential clinical applications of this technique for treatment of cancer. The review reports on the characteristics of microbubbles for drug delivery, the different classifications of microbubbles, approaches to incorporate drugs into microbubbles, effects of microbubbles under ultrasound acoustic pressure, different drug delivery strategies with microbubbles and some limitations of the technology.

Characteristics of Microbubbles for Drug Delivery

The ideal characteristics of microbubbles for potential application as drug delivery carriers are as follows: they should be inert, intravenously injectable, stable during cardiac and pulmonary transit, durable for circulation in the bloodstream, lasting throughout the delivery protocol, detectable and respond in a predictable manner to incident ultrasound acoustic pressure.[14,19,20,25–27] The stability of microbubbles is closely related to the gas content and has been reported that microbubbles with a perfluorocarbon gas core are considered inert and sufficiently stable for circulation in bloodstream during cardiac and pulmonary passage[70] and can be used as carriers of drug molecules to the site of interest.[14]

Classifications of Microbubbles

Microbubbles were originally developed as contrast agents to improve the quality and efficiency of diagnostic ultrasound imaging. In clinical practice, these contrast agents have proven to have excellent safety profiles with no specific hepatorenal toxicity, while reported adverse reactions were generally transient and mild.[15,16,73–75] In general, microbubbles can be classified into three main categories according to the materials that compose its shell: protein, lipid and polymer microbubbles.[14,17] The protein microbubble formulations are commonly made using a thin shell of human serum or bovine serum albumin that surrounds a gas core and are characterised by relatively rigid shells. In contrast, the lipid microbubbles are formed by the self-assembly of phospholipid materials into a thin monolayer shell that surrounds a gas core and the thin shell gives the lipid microbubbles a high flexibility. The polymer microbubbles, on the other hand, consist of thick and bulky shell of cross-linking polymer chains that surround a gas core and make the polymer microbubbles very stable, exhibiting a severely dampened response in an acoustic field. However, some studies[13] have reported a combination of more than one of the above-mentioned materials to form the shell of microbubbles. In general, the lipid and protein microbubbles are considered more attractive drug carriers compared to those of the polymer shell, perhaps due to the thick cross-linked polymer shell which is resistant to fragmentation and thus less effective for depositing genes or drugs.

Incorporating Drugs into Microbubbles

The plausibility of producing microbubbles with different materials is the main advantage for being able to use different strategies to consistently incorporate drugs into the microbubbles and also the compatibility with the type of drug and the delivery site to achieve the optimum therapeutic effect. There are several different approaches by which drugs can be incorporated into microbubbles, include (1) by loading the drug onto the shell of the microbubbles (external and/or internal cavity surface), (2) by embedding the drug inside the shell of the microbubbles using chemical bonds based on avidin–biotin interaction to attach the nanoparticle drug onto the surface of the microbubbles or (3) by using physical interactions of molecules adhesion force that are the result of the exposure by an agitation or sonication field (see Figure 1). Another reported approach of incorporating drugs into microbubbles has been to use electrostatic force interactions (charge coupling) to load the drugs onto the surface of the microbubbles.[13,18] Furthermore, the surface of the shell of the microbubbles can also be functionalised with ligands that specifically bind to receptors at the target area.

The first reported study on the capability to incorporate drugs into the shell of lipid-stabilised microbubbles was in 1998 by Unger et al.[6] They developed an acoustically active lipospheres (AALs), which are similar to lipid microbubbles but contain a thick oil layer separating the lipid shell from the gas core.[6,7] They loaded paclitaxel [(PTX) hydrophobic anti-cancer drug molecules] into the oil layer to create a drug-loaded AAL capable of releasing the contents upon microbubble disruption achieved with ultrasound. The study investigated the lipospheres containing PTX at different concentrations, microbubble sizes and acute toxicities in mice. This initial study on AALs was successful in demonstrating a proof of concept for a novel method of loading hydrophobic drugs within acoustically active bubbles for targeted drug release applications. Shohet et al.[8] investigated the use of albumin-coated microbubbles to effectively deliver an adenoviral transgene to rat myocardium using ultrasound-mediated microbubble destruction approach. They attach adenoviral vectors encoding the *Escherichia coli* β-galactosidase gene onto albumin microbubbles, injected the microbubbles systemically into rats and then exposed the cardiac (chest) region to ultrasound. They reported that, β-galactose expression was observed only in the myocardium following ultrasound-mediated destruction of the microbubbles. This study was one of the earliest proof-of-concept studies that demonstrated the feasibility of organ-specific targeting to deliver viral vectors.

Bekeredjian et al.[19] demonstrated that the luciferase enzyme could be incorporated into a lipid shell of microbubbles for ultrasound-mediated delivery of proteins to the heart. The study further investigated the efficacy of luciferase enzyme incorporated into the shell of lipid microbubbles when injected into rats and then applied ultrasound acoustic pressure. They demonstrated the clinical application of the drugs attached to microbubbles for ultrasound drug delivery using luciferase as a protein model and reported that ultrasound-targeted microbubble destruction can substantially and noninvasively augment organ-specific delivery of proteins. Christiansen et al.[18] performed a study using a bearing cationic lipids that were introduced into microbubbles during formulation and enabled them to electrostatically bind the negatively charged phosphate backbone of plasmid DNA and systemically delivered to rats. The study demonstrated the successful expression of luciferase in hindlimb skeletal muscle and the heart, specifically within the ultrasound-exposed area, following intra-arterial or intravenous infusion of plasmid-loaded microbubbles. This study is one of the first to utilise charge coupling of a lipid microbubble shell with plasmid DNA to improve the specificity of delivery and transfection efficiency. Kheirolomoom et al.[20] described a system of covalently attaching liposomes to lipid microbubbles

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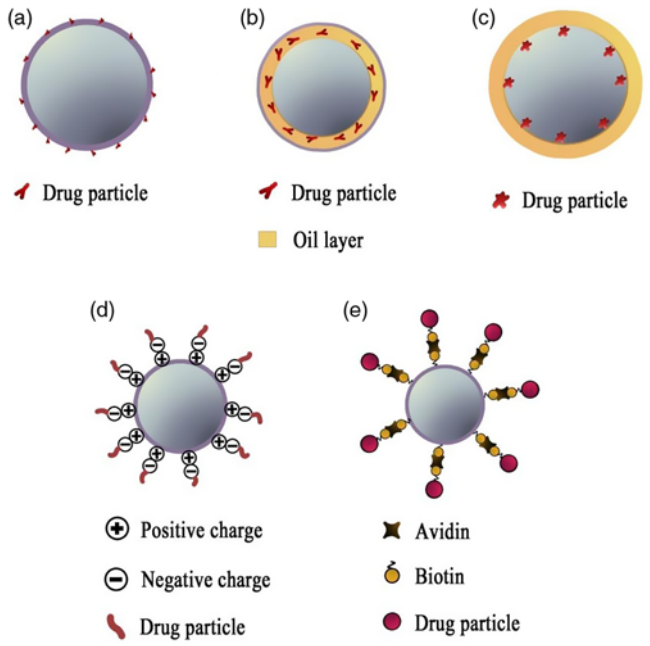

Figure 1. Schematic representation of the approaches of incorporating a drug into microbubbles. (a) Drug loading onto the external microbubble surface. (b) Dissolved drug embedding in an oil layer between gas core and microbubble shell. (c) Drug loading onto the internal microbubble surface. (d) Drug loading outside the microbubble shell through electrostatic interaction. (e) Drug loading outside the microbubble shell through avidin–biotin linkage.

using a biotin–avidin linker system considered to improve the drug-loading capacity by increasing the binding sites available on the microbubble surfaces. In this *in vitro* studies with PC-3 human prostate cancer cell line, they investigated the potential of using fluorescent cholesterol-loaded liposomes as a model of drug molecules for imaging and characterising purposes. They reported a high binding efficiency of liposomes to the microbubble surface and that higher levels of drug loading can easily be achieved even for hydrophobic drugs or proteins.

Shchukin et al.[76] reported a different type of microbubbles' shell that involves polyelectrolyte multilayer (PEM) shells on preformed microbubbles. The preformed microbubbles were coated with a charged surfactant or protein layer, which serves as a substrate for PEM deposition and was considered the first successful attempt of PEM deposition onto microbubbles. Borden et al.[13] also performed in vitro studies using a combination of the PEM technique and electrostatically binding plasmid DNA into the cationic lipid during formulation of microbubbles, giving the microbubbles a negative charge on the surface. Cationic polysine then adsorbed to the shell of the microbubbles, leading to the accumulation of a positive charge on the microbubble surface, which in turn allowed further addition of negative-charge plasmid DNA. As a result of this alternating layers of polylysine and DNA, multiple layers of polyelectrolytes could be formed increasing the DNA loading capacity for targeted gene therapy. Seemann et al.[21] were the first to study the formation of polymer-based microbubbles for gene delivery and studied the encapsulation efficiency and plasmid DNA release behaviour *in vitro*. A double emulsion (water-in-oil-in-water known as in-water drying method) technique was used to form poly(D,L-lactide-co-glycolide) (PLGA) microbubbles containing plasmid DNA complexed to a cationic polymer. The approach used in this study was unique compared to lipid-based and albumin-based microbubbles in that the DNA/polymer complexes can be encapsulated within the PLGA microbubble rather than bound to the surface or incorporated within the shell. There is still ongoing investigation to develop more advanced drug-loading techniques that are aimed at a more effective and efficient binding of the drug onto the surface of the microbubbles until it reaches and is released at the target sites to achieve more optimal therapeutic effect with less toxicities.[24]

Effects of Microbubbles under Ultrasound Acoustic Pressure

The technique by which ultrasound facilitates the delivery of drugs and genes using microbubbles can be achieved by both mechanical and thermal processes that result from a complex interplay among the therapeutic agent, the characteristics of the microbubble, the target tissue and the acoustic energy of the ultrasound. Several studies[14,22–34,71] in an attempt to understand the behaviour of microbubbles under ultrasound acoustic pressure have investigated the different aspects of these mechanical and thermal processes. Dijkmans et al.[25] and Tachibana and Tachibana[24] reviewed the behaviour of microbubbles in the bloodstream and under ultrasound acoustic pressure. They reported that at high transmission acoustic pressure (i.e., large amplitude oscillations), the microbubbles' behaviour become non-linear and induces the surrounding fluid to motion, thereby creating small shock waves that give rise to microstreaming along the endothelial cell. As the acoustic pressure increases, the non-linearity of the behaviour of the microbubbles increases, leading to its collapse that may cause high-energy microstreams (microjets) that will cause shear stress on the membrane of an endothelial cell and increase its permeability. It is further reported that the high-velocity jet streams that are created when microbubbles collapse at high acoustic pressure may cause local transient increase in temperature which affects the fluidity of phospholipid bilayer membranes and could in turn increase the cell membrane permeability.[24,25,30] The increase in permeability is probably due to the transient holes in the plasma

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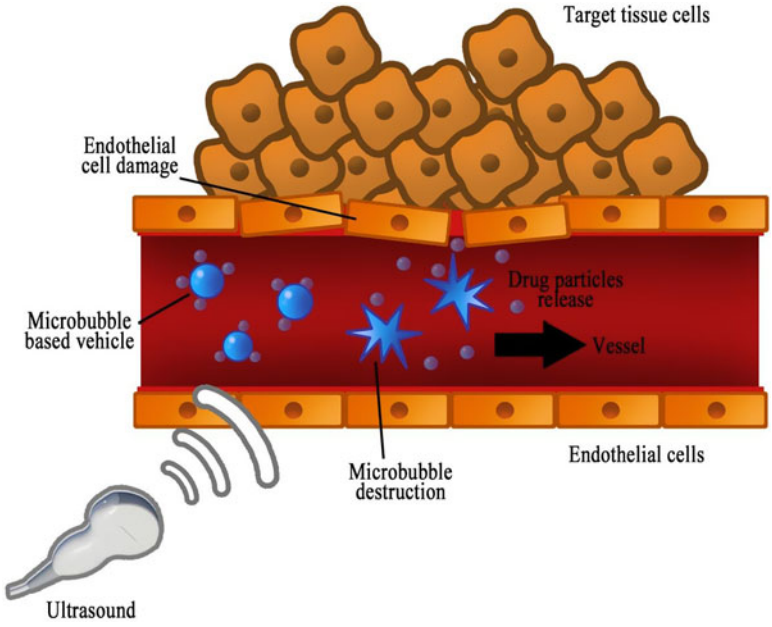

Figure 2. Schematic representation of the effects of applying ultrasound acostic pressure locally on drug-loaded microbubbles. The application may trigger implosion of the microbubbles and causes modification in the permeability of the cells and vessels which allows localised release of drugs at target sites, increasing local drug concentration.

membrane and possibly the nuclear membrane. Taniyama et al.[28] demonstrated the formation of the small transient holes on the surface of endothelial and vascular smooth muscle cells immediately after transfection of a plasmid DNA by collapsing microbubbles in its vicinity with ultrasound. Mukherjee et al.[29] reported that the transient holes on the cell surface are caused by a combination of microbubbles and ultrasound acoustic pressure, which then resulted in a rapid release of the plasma DNA incorporated in the microbubble being deposited into the cell's cytoplasm. Skyba et al.[26] demonstrated that the collapse of microbubbles during ultrasound exposure can cause rupture of microvessels that are ≤7 μm in diameter, resulting in the extravasation of red blood cells (an indication that the collapse of microbubbles can create extravasation points in skeletal muscle capillaries). Price et al.[27] showed that microbubbles with a polymer shell could be driven as far as 200 μm into the parenchyma. According to these studies,[24–27] only a small number of capillary ruptures are required to deliver large quantities of drug particles to a targeted area.

Therapeutic Properties of Microbubbles under Ultrasound Acoustic Pressure

An important therapeutic property of microbubbles, particularly those of albumin shell, is their increased adherence to damaged vascular endothelium. Albumin-coated microbubbles do not adhere to normally functioning endothelium, but they adhere to the activated endothelial cells or to extracellular matrix of the disrupted vascular wall, and this interaction could be a marker of endothelial integrity.[31] As a result of this characteristic, the delivery of drugs or genes bound to albumin-coated microbubbles could be selectively concentrated at the site of vascular injury in the presence[32] or absence of ultrasound application. Porter et al.[33] studied the effect of applying ultrasound on the suppression of intracoronary c-myc protein synthesis within the stent or balloon injury site of pigs using a microbubble delivery system. They intravenously injected anti c-myc bound with a perfluorocarbon containing albumin microbubbles into the coronary arteries of pigs following intracoronary stent or balloon injury. They reported that anti c-myc can be selectively concentrated within an injured coronary artery with similar quantities irrespective of whether the anti c-myc was delivered with the presence or absence of ultrasound. Basta et al.[34] investigated the production of reactive oxygen radicals in endothelial cells under acoustic pressure and suggested an alternate process for the therapeutic effect of microbubbles-mediated drug delivery. Depending on the duration and repetition cycles of exposure to the ultrasound, this can lead to significant increase in intracellular radical production. According to Dijkmans et al.,[25] the combined use of microbubbles with ultrasound can lower the threshold for cavitation and, therefore, could possibly result in an increased production of free radicals, which are associated with cell killing *in vitro* and, consequently, may also be involved in the enhancement of permeability of endothelial cell layers.

Drug Delivery Strategies Using Microbubbles

In general, there are two possible emerging strategies for drug delivery using microbubbles. The first technique of using microbubbles as a 'vehicle' for drug delivery consists of the ultrasound-mediated microbubble destruction, which is based on the cavitation of microbubbles induced by ultrasound acoustic pressure. When microbubbles loaded with drug reaches the target site, ultrasound can be used to collapse the bubbles, which will result in the localised release of the drug (see Figure 2). This drug delivery approach can significantly improve the therapeutic impact of drugs and at the same time reduce its toxicity. There is the potential to be able to use lower concentrations of drugs (to minimise normal tissue toxicity) but achieve the same therapeutic effect to the target using this approach, since the drugs are delivered locally. This is especially useful in cases where the drugs are used regularly but have hazardous systemic side effects (i.e., cytotoxic agents). Furthermore, this approach can induce permeability change on the surfaces of cells and vessels, allowing for the drug delivery in a spatially localised regions. The advantage with this method of drug delivery is that, it can be used in the case of drugs with poor

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bioavailability due to their poor absorption, rapid metabolism and rapid systemic elimination.

An alternate technique of drug delivery using microbubbles is by direct delivery of the drug bonded to the microbubbles (there is no application of ultrasound with this approach). Microbubbles with an albumin shell have been demonstrated to bond to the walls of the blood vessels in the setting of endothelial dysfunction without the application of ultrasound.[31] It has also been demonstrated that perfluorocarbon-filled albumin microbubbles strongly bind proteins and synthetic oligonucleotides,[57] and microbubbles of a phospholipid shell have a high affinity for chemotherapeutic drugs.[7] This approach also has the potential as a drug delivery vehicle to diseased tissues and can possibly be used for different drugs' delivery to target sites. Additionally, the microbubbles can directly take up genetic materials, such as plasmids and adenovirus, where the gene encapsulated or attached to the microbubble is carried to its target site without being eliminated by various enzymes in the body.[57,28] Furthermore, specific ligands for endothelial cell adhesion molecules [such as P-selectin and leukocyte intercellular adhesion molecule 1 (ICAM1)] can be attached to both lipid and albumin microbubbles, which increase their deposition to activated endothelium.[58,59]

Ultrasound-Mediated Microbubble as a Vehicle for Drug Delivery

The application of microbubbles as vehicles for drug delivery first occurred in the early 1990s and since then has led to the emergence of a new era in a technique of targeted delivery of drugs and genes. Numerous investigations[35-40] have confirmed the efficacy of ultrasound-mediated microbubble as a vehicle for drug and gene delivery in both *in vitro* and *in vivo* studies. In clinical oncology studies, the combination of focused ultrasound waves and microbubbles as potential drug delivery vehicles has created an attractive and promising approach for chemotherapy drug deliveries.[39] Current chemotherapy drugs are usually delivered systemically and are therefore typically highly harmful and toxic to healthy cells. Therefore, the potential utilisation of the microbubble approach as drug delivery vehicles for chemotherapeutic drugs (even at high doses) promises direct drug delivery to the tumour cells for targeted therapy, with very minimal systemic toxicities associated with the systemic delivery of chemotherapeutic drugs. Furthermore, the physiological barriers between the interior of blood vessels and their surrounding tissue can limit the delivery of drugs to their intended targets. Nonetheless, the permeability of the walls of the blood vessels can be reversely increased by focused ultrasound, thereby making it possible for drugs to pass through them and to reach the intended tissue.[40] Tan et al.[41] and Teupe et al.[9] and other investigators[42–44] have demonstrated that cell membranes often prevent large drug molecules from entering the cells to deliver their therapeutic effect; however, the absorption of these molecules can be modified and enhanced by taking advantage of the effect of the mechanical force of focused ultrasound through stable cavitation and increased efficacy of drug absorption in precise areas of the body.

The microbubble approach for drug delivery has the potential to induce moderate and reversible changes at the cellular level and to create pores in cell membranes thereby allowing a greater volume of drug compounds to enter cells.[45] Additionally, stable cavitation produces acoustic streaming, which increases the flow of fluid in a cell's environment and may assist in the opening of the pores and can direct therapeutic molecules towards the cells (a phenomenon called sonoporation), thereby enhancing cellular uptake.[46,47] The uniqueness of this phenomenon is that the change in the permeability of the cells' membrane can be temporary and reversible under well-controlled cavitation conditions.[48] The benefit of this unique feature of sonoporation being that, it allows for the restoration of physiological defence mechanisms after successful drug delivery. Enhanced drug delivery via sonoporation could enable the treatment of tumours with dense stroma such as pancreatic tumours, but with less systemic toxicities (i.e., less circulating drug) than with traditional chemotherapy. Focused ultrasound-induced sonoporation is an attractive option for delivery of genetic materials compared to other alternatives of using viruses to deliver genetic materials to cells. This is because it can potentially significantly increase the specificity of treatments[49,50] and the gene therapy can be used to treat a wide range of indications including immunodeficiency disorders, Parkinson's disease, certain types of cancer such as melanoma, ovarian carcinomas, colorectal cancer and hepatocellular carcinomas.[51–56]

Applications of Ultrasound-Mediated Microbubbles in Cancer Therapy

The potential application of microbubbles for drug delivery has induced interest in various medical fields including oncology. The increased interest in this new drug delivery field may be due to its non-invasiveness, local applicability and proven safety in ultrasonic imaging techniques. Several studies[35–38, 60–67] have investigated the potential applications and efficacy of ultrasound-mediated microbubble as a vehicle for drug and gene delivery in both *in vitro* and *in vivo*. Oda et al.[60] investigated the prevention of melanoma lung metastasis using dendritic cell-based immunotherapy. They extracted antigens from melanoma cells that they used to treat the dendritic cells using liposome microbubbles combined with ultrasound and reported a delivery efficiency of about 74%. The dendritic cells treated with the melanoma-derived antigens were assessed for *in vivo* efficacy in a mouse model of lung metastasis, and they concluded that the combination of liposome microbubbles and ultrasound is a promising technique for antigen delivery into dendritic cells. Ji et al.[61] investigated the effect of ultrasound-targeted microbubble destruction of miR-133a (involved in various cancers) on breast cancer treatment in mice model using miR-133a-loaded microbubbles. The study demonstrated high delivery efficiency of miR-133a and reported no significant observable toxicity on alanine aminotransferase and aspartate aminotransferase levels at liver and albumin, blood urea nitrogen or creatine kinase levels at kidney after miR-133a-microbubble injection. They observed reduced tumour size of the miR-133a-microbubble-injected mice compared with the control group and concluded that ultrasound-targeted microbubble destruction of miRNA is a promising technique for breast cancer therapy.

Lentacker et al.[62] demonstrated that doxorubicin (antineoplastic drug) can be delivered to tumour sites as doxorubicin-liposome-loaded microbubbles and with the application of ultrasound. They reported an increase in killing of melanoma cells (more than doubled) after exposure to ultrasound and reduced cytotoxic effect of doxorubicin in non-cancer cells. A clinical case study on patients was conducted by Kotopoulis et al.[63] for the treatment of human pancreatic cancer using combined ultrasound, microbubbles and gemcitabine. The study demonstrated the effectiveness of the combined methodology in decreasing the size of the tumour and prolonging the quality of life in patients with pancreatic adenocarcinoma compared to chemotherapy alone. Liu et al.[64] demonstrated the effectiveness of ultrasound targeted microbubble destruction in the treatment of

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ovarian cancer. They used PTX-loaded lipid microbubbles coated with a luteinising hormone-releasing hormone analogue (LHRHa) through a biotin–avidin linkage to target the ovarian cancer A2780/DDP cells that express the LHRH receptor. They reported a greatly enhanced therapeutic effect of PTX using this technique with high potential in minimising the side effects. Florinas et al.[65] studied both *in vitro* and *in vivo* ultrasound-assisted small interfering RNA (siRNA) delivery via arginine-grafted bioreducible polymer and microbubbles targeting vascular endothelial growth factor (VEGF) for treatment of ovarian cancer. They reported a significantly higher siRNA uptake by tumour tissues, resulting in decelerating tumour growth *in vivo* and VEGF protein knockdown *in vitro* with serum-containing media using this strategy of high therapeutic effects in ovarian cancer treatment.

Kobus et al.[56] investigated a method to improve the response of brain metastases to trastuzumab and pertuzumab (HER2-targeting antibodies) by temporary disruption of the blood–brain barrier of HER2-positive breast cancer patients with extracranial metastases. They used HER2-targeting antibodies combined with focused ultrasound and optison microbubbles in a nude rat model. They concluded that the blood–brain barrier disruption using focused ultrasound in combination with antibody therapy has the potential to inhibit the growth of breast cancer brain metastasis. Blum et al.[66] investigated the effect of nanoparticles formed by acoustic destruction of microbubbles and their utilisation for imaging and effects on therapy by high-intensity focused ultrasound of breast cancer cells. They demonstrated that the exposure of the nanoparticles to the high-intensity focused ultrasound caused breast cancer cells to completely detach from their culture substrate. They reported that subjecting polyethylene glycol-lipid-shelled microbubbles with fluorocarbon interior to ultrasound pulses will potentially produce metastable, fluid-filled nanoparticles that can be re-imaged upon application of high-intensity focused ultrasound. A study by Hou et al.[67] investigated the effect of different low-frequency, low-intensity ultrasound with microbubbles exposure times on prostate cancers and observed changes in the hypoxia-inducible factor 1 and VEGF A protein levels, cell proliferation, apoptosis and tumour volume. They reported that with four repetitions of ultrasound exposure on each treatment day, the cancer cell proliferation was inhibited, apoptosis was promoted and the hypoxia-inducible factor 1α and VEGF factor A expression levels were lower in the treatment group compared to the control group.

Limitations of Ultrasound-Mediated Microbubbles

Considering that microbubbles are administered through the bloodstream, the technique may have some limitations for non-vascular or poorly vascular tissues such as infarct regions, bone and cartilage. Furthermore, bone tissues can strongly attenuate the ultrasound waves. Moreover, the drugs that are carried by microbubbles such as anti-cancer ('antineoplastic' or 'cytotoxic') chemotherapy drugs may face the same challenges as other traditional drugs; that is, the issue of unintentional accumulation of the drugs within healthy tissues. Microbubbles are considered to be too large to extravasate into the cells as they pass through healthy tissue, but over time, these microbubbles and its load of drugs will have a chance to spread further and accumulate in the liver and spleen as these organs perform their natural clearance functions. As these microbubbles degrade, the drugs will be released and accumulated and possibly reach the toxic levels.

Conclusion

The use of ultrasound-mediated microbubbles in the medical field has extended from diagnostic to therapeutic applications and has been shown to have promising application in oncology, thereby becoming the subject of a broad and rapidly developing field of research. Using microbubbles as a tool for drug delivery is considered one of the advanced therapeutic approaches for personalised therapy. Several advantages come with the concept of being able to deliver drug to its pharmacological site of action in a controlled manner, including the protection of the drug against metabolism or recognition by the immune system; the resulting increase in the permeability on cells and vessels using focused ultrasound, thereby allowing for drug delivery in a spatially localised region; and the potential reduction in toxic side effects due to the targeted delivery of the drug. Different explanations have been proposed with regard to the behaviour of ultrasound-mediated microbubbles and its therapeutic effect mechanisms such as transient cell membrane holes, endocytosis, phagocytosis and fusion of microbubble shell components with the cell membrane; however, the exact mechanism remains to be clarified. Although microbubbles used as contrast agents for diagnostic imaging have been shown to be safe, efficient in delivery and approved for clinical use, the toxicity of microbubbles carrying novel drug should be accessed for safety before the translation of the promising technology to therapeutic clinical applications.

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