MICROBUBBLES AS A METHOD OF DRUG DELIVERY FOR TUMOROUS CANCER TREATMENT

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Abstract—This paper discusses the use of microbubbles in the treatment of tumorous cancers and how they can reduce the side-effects and increase the effectiveness of current chemotherapy drugs. The microbubbles are used with ultrasound to focus the treatment at the tumor site, making them a promising technology for targeted cancer treatment. Statistics on the prevalence of cancer along with information on the downsfalls of current chemotherapy techniques are used to clearly demonstrate how the microbubbles impact cancer treatment. Furthermore, it provides an explanation of the composition of microbubbles and how they are used in chemotherapy treatments of tumorous cancers. Additionally, the paper compares the use of microbubbles to the traditional chemotherapy treatments that are used today by comparing and contrasting the effectiveness of the treatments. The paper also demonstrates how ultrasound-assisted microbubble chemotherapy is a sustainable method of cancer treatment. Lastly, it explores the ethics behind microbubble research involving animal testing by weighing the benefits and risks involved.

The main objective of this paper is to increase the awareness microbubble technology and how it can be a more effective and safer method of targeted cancer treatment. Microbubbles have the potential to revolutionize oncology medicine, as well as future applications for targeted drug delivery.

Key Words—animal testing, chemotherapy, microbubbles, tumorous cancer, ultrasound targeted treatment

INTRODUCTION: BACKGROUND FOR MICROBUBBLE TREATMENT OF TUMOROUS CANCER

Cancer affects numerous people in the United States and around the world. Even though cancer is a well-represented disease, the treatment has not yet been perfected. Traditional chemotherapy drugs affect every cell in the body, whether the cell is healthy or tumorous. Gene therapy is another option for treatment of cancer. Gene therapy is currently highly ineffective because genes do not have a long lifespan outside of DNA; they are large nucleotide sequences which are difficult for the cell to uptake. When the genes are not taken up by the cells, they eventually die because the rate of death is very high for genes that have come into contact with the blood stream [3]. Microbubbles are a potential solution to these problems in current cancer treatment. Microbubbles are minute spheres with a hollow center that can be made of various materials. By using microbubbles to target specific damaged cells, the side effects of chemotherapy will be reduced and a lower dosage of chemotherapy drugs will be needed to achieve the same results as traditional chemotherapy. Additionally, utilizing microbubbles in gene delivery protects the genes from the blood stream and allows the genes to be incorporated into the cell in a faster manor than without the use of microbubbles. The microbubble technology also aids in making cancer treatment more sustainable by increasing the cost-effectiveness of the chemotherapy treatments as well as improving the quality of life for those suffering from tumorous cancers. The infinite benefits from using microbubbles as a transport vehicle for genes and chemotherapy drugs make them a favorable solution to current chemotherapy and gene delivery complications while also increasing the sustainability of chemotherapy treatment.

CANCER AND CHEMOTHERAPY DEMOGRAPHICS

There are over 100 different types of cancer [1]. Men in the United States have “slightly less than a 1 in 2 lifetime risk of developing cancer; for women, the risk is a little more than 1 in 3” [1]. However, certain individuals can have a higher or lower risk due to factors such as gender, tobacco use, and genetics [2]. Cancer affects a broad spectrum of people and is the second leading cause of death in the United States, after heart disease [2]. In the United States, approximately 1 in every 285 children is diagnosed with cancer before the age of 20 [2]. In 2014, it is predicted that, “about 1,655,540 new cancer cases are expected to be diagnosed excluding carcinoma of any site except urinary bladder, nor does it include basal cell or squamous cell skin cancers” [2]. This statistic is comparable to over two-and-a-half times the population of Washington D.C. being diagnosed with cancer. With so many people being affected by this deadly condition, one would infer that the survival rate is relatively high but “the 5-year survival rate for all cancers diagnosed between 2003 and 2009 is 68%” [2]. Technology to treat cancer is evolving, however, it has not been perfected and it will take many years before the perfect solution can be found.

Current treatments for cancer include surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy [3]. One of the most popular treatments for cancer is chemotherapy. Chemotherapy interferes with the cancerous cell’s ability to grow or reproduce [4]. The drug enters the body through oral intake, intravenously, or injection. This allows the chemotherapy drug to move freely...
throughout the body, producing side effects that can potentially be deadly. “There are over 50 chemotherapy drugs that are commonly used,” which evoke various side effects. These side effects include hair loss, nausea, vomiting, diarrhea, kidney damage, bladder damage, mouth ulcers, heart damage, seizures, joint pain, hearing loss and many others [4]. Most of these side effects are due to the chemotherapy drug being processed by the whole body and not just by the cancerous cells. By targeting the cancerous cells, the side effects of chemotherapy can potentially be decreased or even eliminated.

**CHARACTERISTICS AND COMPOSITION OF MICROBUBBLES**

Microbubbles have many advantages for the treatment of tumorous cancer because of their characteristics and properties. Most microbubbles are the same size as a red blood cell, which makes them an ideal packaging material. They are also small enough to allow for passage through all areas of the body with minimal resistance [5]. Microbubbles expand and contract due to the pressure from an ultrasound machine. How the bubble reacts depends on the material of the shell, frequency of the wave from the ultrasound and other factors. Proteins, lipids, and polymers are the common materials used to form microbubbles [5]. Each material has different benefits and downfalls.

**FIGURE 1 [5]**

Microbubble with a section view of the shell with lipid, protein and polymer material

**Lipid Based Microbubbles**

Lung surfactant, the hydrophilic and hydrophobic region of the lung’s surface, is the model from which lipid microbubbles were formed [6]. The stability and compliance of lung surfactant made it the best model to sculpt microbubbles from. Making lipid microbubbles is a fairly simple process since the phospholipids spontaneously orient to form a monolayer at the air-water interface, as illustrated in Figure 1 [6]. Lipid shells for microbubble use are successful due to the physical properties of lipids. The hydrophobic acyl chains that face the interior of the microbubble keep the drug inside the bubble until lysis [5]. This allows for maximum drug delivery to the site of which the drug is intended, in most cases a tumor. The hydrophilic (“water loving”) head-groups face the exterior of the bubble which allows for easy movement through the body and makes them impermeable to other hydrophilic molecules that could dilute the drug inside [5]. Hydrophilic and hydrophobic properties of lipid based microbubbles encourage maximum drug delivery to the site instead of a slow release.

Lipid microbubbles are also held together by weak physical forces that can tolerate the compression and expansion of the microbubbles without much influence from an ultrasound [5]. These weak forces also permit lysis, or rupture, of the microbubble at a lower frequency than other materials as well as the ability to see the bubbles traveling through the body using ultrasound. Several lipid based microbubbles are approved for clinical use in the United States and abroad, however they are not specifically for use in cancer therapy [5]. Lipid microbubble material is seen as a “versatile platform technology” since it can be adapted to perform numerous tasks with little alteration [5].

**Protein Based Microbubbles**

Proteins are one of the oldest materials used to form microbubbles. The first protein based microbubbles to be approved by the United States Food and Drug Administration was Albunex, which and was used as a contrast agent, to increase clarity in ultrasound pictures of the left ventricle of the heart [5]. Protein based microbubbles are produced by denaturing the protein and then putting the proteins through a process called sonicication, which is when ultrasound is applied to induce a desired activity in materials. The proteins undergo sonicication with air, which results in encapsulation of air within the shell of the protein [5]. The process is used to make Albunex and in one milliliter of solution there are $7 \times 10^8$ microbubbles present, which suggests that process makes mass production of the microbubbles fairly easy and efficient [5].

**Polymer Based Microbubbles**

Plasma polymer microbubbles are “nonconventional solids that can form nanoparticles as well as hollow nanoshperes” [7]. These microbubbles are formed by cross linking or entangling polymers at the air-water interface [6]. This allows for an outer wall of polymer material and a hollow center as illustrated in Figure 1. The hollow polymer spheres “have radially uniform shell thickness of $47 \pm 18\text{nm}$ and a high void fraction that is $90\pm3\%$ of the volume of the particle [7]. Polymer microbubbles are made by producing solid bubbles that expand to twice the original size, which produces a hollow cavity. The hollow cavity can contain various materials like gas, chemotherapy drugs, Small-Interfering-RNA (siRNA, RNA that interfere with expressions of genes), and other therapy materials. Polymer
based microbubbles are generally used for “controlled release because of the controllability over their size, hydrophobic properties and their mechanical properties” [7]. Controlled release, which is when a drug is released in a specific manner dependent on time or location, is possible by controlling the properties of the polymer. For example, the bubble may slightly fracture on purpose, divide into sister cells, or allow a certain amount of material through the shell at a time [7]. Generally polymer shells resist compression and expansion more than lipids and proteins, which make it possible for the polymer to fracture resulting in an uncontrolled release [7]. Polymer microbubbles have benefits and downfalls but are an effective shell material for various uses.

VARIOUS USES OF MICROBUBBLES AND HOW THEY CAN BE USED FOR TUMOROUS CANCER

Microbubbles have various applications to the medical field. Opening clotted arteries, delivering drugs, contrast for ultrasound and other activities are possible with microbubbles [11].

Ultrasound Molecular Imaging

Ultrasound molecular imaging is very similar to basic ultrasound imaging. The main difference is that ultrasound molecular imaging done on the molecular level, and typical ultrasound imaging is done on the tissue level. The microbubbles must be adapted for this more specific imaging. The concept of ultrasound molecular imaging is to “selectively adhere ligand-bearing contrast agent particles to endothelia expressing a target receptor and, by virtue of contrast agent accumulation and echo enhancement, to map and quantify the extent to which vasculature express that target receptor” [5]. Figure 2 illustrates this idea that the microbubble has a ligand on the exterior, which then attaches to the receptor site in the targeted cell. It follows the idea of a lock-and-key mechanism. The ligand is the key and fits into the receptor site, which acts as the lock.

The microbubbles now target certain cells and adhere to those cells because of the ligand and receptor pair. More sound waves then deflect from the targeted image site, since the microbubbles are attached to the cell and expand and compress due to the ultrasound [5]. Molecular ultrasound imaging with targeted microbubbles allows the tumor to be quantified more precisely. Ultrasound molecular imaging with microbubbles allows the viewer to attain more exact dimensions of the area that the tumor affects. Knowing exact tumor size allows the physician to administer a more exact amount of chemotherapy drug to treat the tumor. This makes microbubbles sustainable because there is less chemotherapy drug wasted. This is possible since the microbubbles deflect more sound waves from the ultrasound [5]. This causes decrease the number of ultrasound waves that pass through each layer, which allows a clearer picture of the tumor [5]. If medicated microbubbles are used in conjunction with gas filled microbubbles, one can see the medicated microbubbles disappearing. The gas microbubbles must be made of a different material than the medicated; otherwise they will rupture at the same time, and the gas microbubbles will not produce a clearer picture [5].

FIGURE 2 [8]

Targeted microbubbles for ultrasound molecular imaging

Gene and Drug Delivery

One of the most researched microbubble applications is gene and drug delivery. There are many advantages to microbubble transport vehicles for gene and drug therapy. Microbubble cavitation allows permeability of the endothelial vasculature (cell lining the interior surface of blood vessels and lymphatic vessels), which clears a path to allow larger molecules to reach the tumorous cells with less difficulty, this is illustrated in Figure 3 [5]. The process of increasing the permeability is known as sonoporation [5]. This makes the uptake of siRNA, DNA and other macromolecules by the cell easier because the molecules are typically too large to enter through the normal cell pores. The sonoporation causes large pores to form, which makes it easier to larger molecules to pass through the cell membrane [5]. Tumorous cancer is treated either by gene or chemotherapy drug administration. Chemotherapy drugs are typically small molecules, but genes are large macromolecules. Sonoporation enables the macromolecules, like genes, to enter the cell and be incorporated into the DNA more rapidly. Microbubble oscillation near the cell membrane can cause hyperpolarization, which will allow macromolecules to be taken into the cell easier, like sonoporation [5].
Another benefit of using microbubble vehicles to transport genes and drugs into cells is the rapidity that the payload of the microbubble is released [5]. When introduced to the bloodstream DNA degrades easily, but when encapsulated in a microbubble DNA degrades less readily and improves the specificity of delivery [5]. Sonoporation allows faster uptake of the DNA or siRNA into the cell’s existing DNA, which then allows for transcription in a shorter amount of time than without sonoporation. Since DNA and siRNA have a relatively short lifespan, the faster uptake and transcription increases the chance that the DNA or siRNA will still be able to alter the cell’s existing DNA.

**FIGURE 4 [8]**

Various methods of incorporating DNA, siRNA, or chemotherapy drugs into/onto microbubbles

Microbubbles are an advantageous vehicle choice for gene and drug delivery, but the one major problem is the loading capacity of the bubbles. Since the bubbles are relatively small, the amount of genes and drugs that can be encapsulated by the bubble is minimal [5]. Another obstacle is how to incorporate the therapy to the microbubble. Image A in Figure 4 illustrates therapy uptake by the microbubble, which is the most popular method of therapy uptake. Image B in Figure 4 delineates incorporation of the therapy into the membrane of the microbubble. Image C in Figure 4 highlights attachment of the chosen therapy to the exterior of the microbubble. Attachment to a ligand is portrayed in image D in Figure 4. Incorporation of the selected therapy to the membrane of the microbubble is depicted in image E of Figure 4. Microbubbles are still being heavily researched and innovated to improve various aspects to be more suited for different tasks, which makes microbubbles a technology that has the capability to evolve to meet desired specifications in the future.

**MICROBUBBLES VERSUS TRADITIONAL CHEMOTHERAPY TREATMENTS**

Current chemotherapy techniques kill cells regardless of whether they are healthy cells or tumorous cancer cells [9]. An article by researchers at the Moores Cancer Center in La Jolla, California stated that in traditional chemotherapy, drugs are injected into the circulatory system and are allowed to circulate throughout the body, while very little of the drug actually makes it to the tumor site and attacks the tumor cells [9]. Any drug that does not make it to the tumor site is then left to circulate within the body, therefore the drug is able to affect healthy tissues and cells [9]. The excess drug can then produce undesirable side-effects, such as nausea, vomiting, hair loss and bone marrow depression [10].

The same article by the Moores Cancer Center researchers described the main goal of chemotherapy research as finding a method of delivery that would decrease the aforementioned side-effects of chemotherapy drugs and increase the payload of potent cytotoxic drugs to the tumor sites in a controlled manner [9]. Drug-loaded microbubbles that are triggered by ultrasound have the necessary properties to provide these desired characteristics of the ideal chemotherapy treatment. These properties include containing the drug to protect the healthy tissues, rapidly depositing the drug to the tumor site, circulating within the body for the necessary amount of time to reach the site, and allowing the drug to penetrate the cell [9]. By decreasing the amount of undesirable side-effects by targeting only the tumorous cells, the microbubble technology would be able to sustain or even increase the quality of life of cancer patients. By eliminating unnecessary and undesirable side-effects the emotional and physical well-being of the patients will be improved. The emotional trauma such as the psychological impact of losing one’s hair, and the physical ailments, such as decreased immune function, could potentially be eliminated.

Another advantage of microbubble cancer treatments, according to Dr. Flordeliza Villanueva, director of the Center for Ultrasound Molecular Imaging and Therapeutics
Gabrielle Schantz  
Karaz Horbach

at UPMC Presbyterian, is that they can increase the cell permeability of genetic and chemotherapy drugs through sonoporation as well as providing a targeted treatment that will reduce undesirable side-effects of chemotherapy drugs, such as doxorubicin, paclitaxel, and 10-Hydroxycamptothecin [11]. The increases in cell permeability can also increase the effectiveness of the chemotherapy treatment by allowing more of the drug to enter the cell and increase the rate of tumor cell death, while also decreasing the rate of tumor cell proliferation [11].

Furthermore, ultrasound-triggered microbubble therapy can be easier to administer than traditional chemotherapy, which can involve multiple injections to the tumor site [11]. These injections can be painful and do not uniformly distribute the drugs throughout the tumor. The non-uniform distribution of the chemotherapy drug then causes the treatment to be repeated multiple times, which is not always feasible [11]. Microbubbles have a safer, non-invasive, and less painful administration process due to the fact that they are introduced to the bloodstream through traditional intravenous therapy [11]. Microbubble treatments also take advantage of traditional ultrasound machines to directly focus the target on the tumor, which increases the uniformity and concentration of drug infiltration [11]. By increasing the uniformity, the need for successive treatments is greatly reduced. The decreased need for successive treatments benefits the patients by eliminating the need for multiple trips to cancer treatment facilities sustains the cost-effectiveness of the treatment for the patients.

Since treatment is administered with technology already used in medicinal practice, there is no need to develop new machinery. This allows the process to be both more beneficial and cost-effective to both the hospitals providing the treatment and the patients receiving it, which in turn aids in the sustainability of chemotherapy treatments.

Alongside the easier administration process, microbubble treatments have been proved to be more effective at combating tumorous cancer cells than traditional chemotherapy administration due to the targeting effects of the ultrasound frequencies [12, 14, 15]. The increased effectiveness sustains the amount of chemotherapy drugs available for use by decreasing the amount of wasted drug that does not reach the tumor site as well as increasing cost-effectiveness of the chemotherapy treatment, which reduces financial burden of those living with cancer.

THE EFFECTIVENESS OF VARIOUS MICROBUBBLE TECHNOLOGIES FOR CANCER TREATMENT

Despite their similar structures, each type of drug-loaded microbubble will produce different rates of effectiveness at treating cancerous solid tumors. In order to understand the ways in which these results differ, the following sections will explain the various microbubble drug delivery systems that are currently undergoing research and how effective they are. Additionally, we will compare and contrast microbubble technologies to current chemotherapies to demonstrate how effective microbubbles are at combating cancer.

Doxorubicin-Liposome Microbubble Complexes

Not only are the types of treatment unique in chemotherapy, the tumors that they target are unique as well. In some cases, the tumors contain a multidrug resistant phenotype that can cause subsequent exposure to a drug to be ineffective. This can be seen in the treatment of human breast cancer tumors. Doxorubicin-liposome microbubble complexes (DLMC) could potentially reverse this phenotype to allow successive chemotherapy treatments to be effective [12]. A study on the reversal of this phenotype in human breast cancer cells demonstrated that, “the viability of cells treated with DLMC + US (ultrasound) containing 5 micrograms/milliliter of DOX (doxorubicin) was significantly lower, achieving 58.3 ± 1.8% compared to DL (doxorubicin liposomes) +US (85.5 ± 1.4%)” [12]. The researchers believed that sonoporation caused the released DOX to enter the resistant drugs and accumulate in the cell nucleus, disrupting the cell DNA and causing a decrease in cell viability [12]. This demonstrates that DLMCs can be an effective means at treating breast cancer tumor cells. However, the study did state that in vivo testing is needed to understand how the internal environment of the bloodstream would affect the efficacy of the DLMC +US systems [12]. Once research is done on how the internal environment affects the efficacy of the DLMCs, researchers will be able to determine if the drug delivery system will be effective in treating of tumorous cancers that have expressed multidrug resistance and can no longer be treated with certain cancer drugs that would be potent and cytotoxic to the tumors.

Paclitaxel-Liposome Microbubble Complexes

Paclitaxel is another powerful chemotherapy drug that has been used for the treatment of solid tumors such as breast cancer, but its use has been limited due to its low solubility. Paclitaxel-Liposome Microbubble Complexes (PLMC) can provide an alternative for toxic solutes used to administer traditional paclitaxel treatments and provide a more targeted dosage of paclitaxel in tumorous cancers [13]. A study performed on the usage of PLMCs as ultrasound-triggered therapeutic drug delivery carriers at the Paul C. Lauterbur Research Center for Biomedical Imaging in Shenzhen, China resulted in a 3.54 and 4.31-fold higher Paclitaxel accumulation in tumors when Paclitaxel microbubble-complexes were combined with ultrasound in tumors in comparison to paclitaxel microbubbles without ultrasound and paclitaxel injections [14]. The higher accumulation of Paclitaxel inside the cells allow the drug to
work more efficiently and effectively at destroying the cancer cells. This increase in cell death was quantified by a reduction of tumor volume by approximately 360.01 ± 131.24 mm$^3$ in tumors treated with PLMCs and ultrasound [14]. Another study on Paclitaxel-loaded microbubbles resulted in a decrease in cell proliferation of the tumors to 36.77 ± 32% and a cellular-uptake that was 7.66-fold that of non-targeted microbubbles at 120 seconds [10]. PLMCs show promising results in being more effective than traditional paclitaxel treatment of tumors by decreasing cell proliferation and cell volume. This suggests that not only will PLMCs slow the tumor growth process; they can potentially destroy and eliminate the tumor cells. Additionally, since the same drug was used the increase in effectiveness can be correlated to the unique properties of microbubbles that are exposed to ultrasound.

10-Hydroxycamptothecin-loaded Phospholipid Microbubbles

The chemotherapy drug 10-Hydroxycamptothecin (HCPT) has powerful anti-tumor qualities and is currently used in the treatment of a wide variety of tumors. Additionally, the required dosage is over twenty times lower than paclitaxel and a doxorubicin, which makes it a very viable option for microbubble technologies, which tend to have low drug payloads [15]. The 10-Hydroxycamptothecin can be loaded into phospholipid microbubbles and triggered by ultrasound to target the drug within a tumor. Furthermore, the tumors exposed to 10-Hydroxycamptothecin Loaded Phospholipid Microbubbles (HLMs) and ultrasound had a 5-fold higher HCPT concentration in tumor tissues than HLMs without ultrasound (54.5 ± 8.2 ng vs. 10.4 ± ng per gram of tissue) [15]. Increasing the accumulation of the drug within the cell leads to a higher cytotoxicity rate, which would increase the effectiveness of the drug in comparison to 10-HCPT administered without the microbubbles [15]. The lower dosage needed conserves the amount of the drug needed to be made for use as well as limiting the undesirable side-effects, such as hair loss, which increases the emotional and physical qualities of life for those suffering with tumorous cancers [15]. Additionally, this high payload and low dosage may make HLM treatment more advantageous than the previously mentioned PLMCs and DLMCs; this would then make 10-HCPT filled microbubbles aided by ultrasound technology the most sustainable form of microbubble cancer treatment.

**DRAWBACKS OF MICROBUBBLES IN CANCER THERAPY**

Microbubbles have many advantages over traditional chemotherapy techniques; however they are not perfect and have disadvantages as well. Despite a decrease in the amount of off-site side effects through the usage of microbubbles, the potential for healthy tissues to be affected by the chemotherapy drugs is still present. The chemotherapy drugs can still accumulate in the liver and spleen due to the microbubbles becoming trapped and slowly disintegrating overtime [9]. In order to find out more information on the toxicity of microbubbles, we met with Dr. Flordeliza Villanueva of UPMC Presbyterian, who has been researching microbubbles as chemotherapy and gene therapy utilizing microbubble technology and ultrasound frequencies. During our meeting with Dr. Villanueva, we discussed the drawbacks of microbubbles in her research. One major detractor of microbubble technology is that the dosages of microbubbles used on the murine test subjects would be toxic if scaled up to the amount needed for human treatment [11]. The mice do not die because the gas concentration is low enough to not effect gas levels in their bloodstream. This presents the challenge of maximizing drug payload within the microbubbles in order to decrease the amount of microbubbles that have to be injected into the bloodstream.

Another downside of the current research on microbubbles is the length of time it will take until the technology is ready to be used in hospitals and cancer treatment centers. The cost and time needed for the toxicology and pharmacology at the preclinical stage can limit the rate at which research progresses [11]. Furthermore, the clinical trials that will be needed in the future will also take a substantial amount of time and increase the cost of research, both of which will lengthen the amount of time until the technology can be utilized in the clinical treatment of tumors, such as breast cancer [11]. Additionally, clinical trials have not yet started and the efficacy rates are dependent on animal models in in vivo testing rather than human models [11]. This can lead to skewed results and predications about how the technology will work inside the human body. Furthermore, there are some ethical issues associated with using the animal models rather than human models in testing the technology. Such as the humane treatment of the animal test subjects, the reliability of animal test results, and the sustainability of the animal testing.

**ANIMAL TESTING IN MICROBUBBLE CANCER THERAPY RESEARCH**

In order to determine the efficacy and the potential risks associated with the different microbubble technologies, many research teams utilize animal test subjects, in most cases mice, to test how the internal environment affects the effectiveness of the microbubble drug delivery systems. Typically, the mice are injected with some form of cancerous cells, which are allowed to grow to a desired diameter or volume. This process was done by members of the Chongqing Medical University who purposely injected...
mice test subjects with tumorous cancer cells on the dorsal flank area in order to test the effectiveness of the 10-Hydroxycamptothecin-loaded microbubbles previously mentioned, which can be seen in Figure 5 [15].

**FIGURE 5 [15]**

<table>
<thead>
<tr>
<th>Saline control</th>
<th>Unloaded MBs</th>
<th>HCPT injection</th>
<th>HLMs</th>
<th>US + HLMs</th>
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<tr>
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<td><img src="image2" alt="Unloaded MBs" /></td>
<td><img src="image3" alt="HCPT injection" /></td>
<td><img src="image4" alt="HLMs" /></td>
<td><img src="image5" alt="US + HLMs" /></td>
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**Mice Test Subjects with Tumors and Tumors Removed after 10- Hydroxycamptothecin Microbubble Treatment**

The tumors were allowed to grow to 0.5 centimeters before any tests occurred. Four hours after the microbubbles had been administered, the mice were then sacrificed and the tumors excised for examination [15]. This process complied with the Animal Ethical Commission of Chongqing Medical University and in the study the mice were under anesthesia during the procedure to minimize discomfort [15].

This process can be consider inhumane and cruel due to the fact that the mice are purposely injected with cancer and later killed in order to determine the efficacy of something that will be used on humans. However, the trials are necessary to determine how the drugs will react internally, and will provide results that could benefit both people and animals suffering from cancer.

The reason for using animal test subjects in *in vitro* testing was summarized by the researcher who studied the effectiveness of PLMCs. The researchers claimed that the *in vitro* environment “does not capture the complexity of the *in vivo* situation. Factors such as tumor vessel or lymphatic vessel diameter, blood speed, and wall shear stress possibly affect the attachment of targeted drug-loaded (microbubbles)” [10]. The *in vitro* cell cultures used to test the microbubble drug-delivery systems lack the aforementioned variables, which makes it difficult to predict how the technology will react in the environment that they are intended to be used in. Animal models are used because they can more closely mimic the internal environment of the human body than cell cultures can. However, there can be problems associated with using animal models over using humans as test subjects.

A major problem associated with animal models is that between species of mammals, the “anatomy, metabolism, physiology or pharmacology caused by underlying genetic, variations,” differ to some degree [16]. According to a review of various research articles on the limitations of animals there are, “at least 67 known discrepancies in immunological functions between mice and humans” [16]. This leads to the question of whether or not the studies of microbubble efficacy are accurate and reflect what would happen in the human body. However, the studies are used for preclinical research to determine whether or not the technology is worth pursuing in clinical trials, which can be more time consuming and more expensive [11]. The promising results suggest that clinical trials may be an option for the research.

Additionally, animal testing can both hurt and aid sustainability. It hurts sustainability by needing multiple test subjects who are eventually euthanized because the research involves studying their internal anatomy. However, it aids sustainability because the animal testing is both more time-efficient and cost-effective than full-scaled clinical trials with humans. It also prevents resources from being used wastefully and recklessly by determining whether the study is worth doing clinical trials.

**CONCLUSION: MICROBUBBLES A PROMISING CHEMOTHERAPY TREATMENT**

Overall, microbubble usage in chemotherapy techniques proves to be a more effective and safer method of drug delivery than currently used chemotherapy delivery systems. The unique properties of microbubbles allow them to be transferred to specific tumor sites within the body through the bloodstream and only release the drugs when they are exposed to the necessary frequencies of ultrasound. Additionally, this targeted approach of chemotherapy delivery will decrease the number and severity of undesirable side-effects associated with potent chemotherapy drugs, such as Doxorubicin and Paclitaxel.

Microbubble technology also has the potential to increase the uptake of the chemotherapy drugs by the tumor cells, which in turn increases the effectiveness and the rate at which chemotherapy drugs attack and destroy cancer cells within tumors. The microbubble technology also proves to be more sustainable than current chemotherapy techniques by utilizing current ultrasound technology as well as increasing the quality of life for tumorous cancer patients. However, this research is far from perfect and even farther from being used commercially in cancer treatments because of the obstacles that need to be overcome. Such obstacles include maximizing drug payload and determining ways to keep the microbubbles from accumulating in the liver and spleen of test subjects. Once the major problems associated with current microbubbles are addressed, the technology has the potential to cause a revolutionary approach to chemotherapy treatment of tumorous cancers.
REFERENCES


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