OPTIMAL ULTRASOUND PULSE PARAMETERS
FOR DRUG DELIVERY

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ABSTRACT

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By Nandini Gurunathan, Trupti Partani and Varun Verma

The field of localized drug delivery has been a topic of interest for several decades. The ability to cure a disease at its origin while minimizing or avoiding systemic side effects due to conventional treatment procedures is a lucrative proposition. This has now evolved into an achievable feat with the advent of microencapsulation technologies. The use of microencapsulation techniques to deliver drugs to specific sites in the body has great potential, especially in the case of breast cancer treatment. Our project focuses on microencapsulating chemotherapeutic drugs and ultrasound contrast agents within large biodegradable polymeric microcapsules. These microcapsules can then be coupled with therapeutic ultrasound to enable localized delivery of the drug at the site of the breast tumor. The main aim of this project is to identify the ultrasound parameters such as frequency; intensity and pulse width at which optimal drug delivery can be achieved. The changes in the drug elution rate due to the manipulation of these parameters are also studied. Our project report will conclude with an analysis of the future prospects for this technology.
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Nandini Gurunathan
Trupti Partani
Varun Verma
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LIST OF ABBREVIATIONS

UCA – Ultrasound contrast agents
ACS - American Cancer Society
MHz – Mega Hertz
W/cm² – Watt per square centimeter
NaCl – Sodium Chloride
CaCl₂ – Calcium Chloride
AIUM - American Institute for Ultrasound in Medicine
μm – Micrometer
DI water – Deionized water
R & D – Research and Development
mW/cm² - milli watt per square centimeter
FDA – Food and Drug Administration
mVpp- milli volt, peak to peak
Sec- Second
CHAPTER 1: INTRODUCTION

Developing new modes of drug delivery has been a field of constant research since most currently available drug delivery mechanisms are coupled with side effects. A study by Wang et al., in 2004 specifies that an ideal drug delivery system must be capable of reducing the degradation of the drug, positively manipulating the kinetics and bio-distribution of the drug, providing a controlled or sustained drug release, minimizing harmful effects, and improving the
drug specificity, leading to drug efficacy, safety and patient compliance. Our project aims to achieve this by developing an ideal drug delivery mechanism for the treatment of breast cancer.

Our project is based on the use of microcapsules, which are hollow spheres that carry a chemotherapeutic drug and several minute gas bubbles called ultrasound contrast agents (UCAs). These drug carrying microcapsules will be injected to the site of the breast tumor and be exposed to a therapeutic ultrasound beam. Upon exposure, the UCAs within the microcapsules will undergo excitation by means of oscillation or vibration and emit secondary ultrasound waves in all directions. As a consequence, the microcapsules will undergo a change in membrane permeability and lead to the elution of the drug contained within them. This technology will thus enable a controlled mechanism of drug delivery within the body that can be triggered by periodic intervals of therapeutic ultrasound application. The prime focus of our project is to determine the therapeutic ultrasound conditions at which this can be achieved.

To gain a better insight into this topic, an introduction to the prevalence of breast cancer and drug delivery mediated by therapeutic ultrasound is required. These topics are briefly discussed in this chapter.

1.1 BREAST CANCER – SIGNIFICANCE OF THE PROBLEM:

Cancer is the second largest cause of death in the US, accounting for 23.1% of all deaths in 2009. Breast cancer is widespread in the United States. According to estimates from the American Cancer Society (ACS), breast cancer was the second largest type of cancer among women that proved fatal to 15% of the female cancer population in 2009. From 2002 - 2006, breast cancer has had an incidence rate of 123.8 per 100,000 women per year as seen in the figure below. In 2008, ACS has estimated that a staggering 194,280 cases of invasive breast
cancer will occur in 2009 causing 40,610 cancer-related deaths. In addition, 62,280 new cases of
\textit{in situ} breast cancer were also expected to occur in women in the US alone.

![Fig. 1 Cancer incidence rates among US women (1975 – 2005)](image)

Even though breast cancer incidence rates remain high, the fatality of the disease has reduced due to conventional treatment procedures which are effective but crude. Breast cancer treatment depends on the stage of the disease. Staging is done on the basis of tumor size, lymph nodal status (to determine if the cancer has spread to the lymph nodes) and metastasis (to determine if systemic spread of the cancer has occurred).
In the initial stage, when a small tumor is present locally (*in situ*) in the breast, it can be treated by surgery. This can involve the removal of the entire infected breast by a procedure termed mastectomy. Another option is a breast conservation surgery, which involves a minimally invasive procedure to remove the tumor and some of the surrounding tissue. These procedures are usually followed with adjuvant therapies like radiation therapy or chemotherapy to prevent occurrence of secondary cancers.

![Fig. 2 Treatment options for Breast Cancer](image-url)
If the disease has progressed to a later stage where the lymph nodes surrounding the breast is affected and the tumor is much bigger in size, the treatment procedure would involve a breast surgery and a lymph node surgery, followed by adjuvant therapies. In the last stage of the disease, metastasis of the disease would lead to systemic spread of the cancer. At this stage, the disease is difficult to cure and requires multiple surgeries and other adjuvant therapies.

Although cancer affects only the breast in the initial stages of the disease, the conventional treatment procedures involve crude procedures and adjuvant therapies which have systemic effects that can affect the whole body.

- Breast cancer surgery can cause lymphedema (swelling of arm or hand due to fluid accumulation) and neutropenia (decrease in white blood cells) in some patients.
- Radiation therapy uses high-energy rays to stop the proliferation and to kill the cancer cells. It can cause fatigue, damage to skin and make the breast heavy and tight.
- Chemotherapy causes side effects like digestive tract causing infections, hair loss, poor appetite, nausea and fatigue. It can also affect the ovaries and cause infertility.
- Hormone therapy can lead to nausea, mood swings, depression, weight gain, blood clots (thrombus) and uterine abnormalities.

To avoid these side effects associated with the treatment, there is a constant search for new treatment options that can improve the efficiency of the treatment and minimize the side effects associated with the treatment.

Our project focuses on this need for a new treatment technique with the use of therapeutic ultrasound which uses non-ionizing energy to modulate drug release from drug carriers like microcapsules.
1.2 ULTRASOUND MEDIATED DRUG DELIVERY:

Recent studies by Crum et al. (2010) show that therapeutic ultrasound is capable of sonothrombolysis (removal of blood clots), shock wave therapy (treatment of pain with high-intensity acoustic radiation), tumor ablation and site-specific drug delivery. Further studies on site-specific drug delivery show that with the use of therapeutic ultrasound it is possible to penetrate deep into the body to deliver mechanical or thermal energy to a specific site accurately without damaging surrounding tissues (Curra and Crum, 2003). Based on this principle it is apparent that therapeutic ultrasound has a great potential for use in targeted and controlled drug delivery.

An early application of ultrasound in drug delivery was reported in curing polyarthritis by facilitating efficient uptake of the drug into infected tissues (Ng and Liu, 2002). Experimental studies also showed that therapeutic ultrasound could be used for the successful administration of anesthetic drugs across the skin (Mitragotri, 1991). The important aspects of ultrasound-mediated drug delivery, as seen in these applications, are to attain location specificity, increase the drug uptake and to decrease the systemic side effects of the drug.

Initial drug delivery experiments using ultrasound was done at high intensities which lead to tissue heating and tissue damage at the site of application limiting the use of ultrasound. However, this was avoided with use of low intensity ultrasound and ultrasound contrast agents (UCAs). UCAs are tiny gas filled microbubbles that absorb the low intensity ultrasound energy applied and amplify it by emitting secondary ultrasound waves, thus producing results like those produced by high intensity ultrasound while preventing tissue damage (O’Brian, 2007).

Researchers have shown that drug delivery to solid tumors in a specific and targeted manner was often inefficient thus resulting in toxic effects of drugs in the surrounding tissues.
The use of ultrasound as a potential treatment option for cancers and tumors gained impetus owing to these harmful side effects that resulted from conventional treatment options like chemotherapy, radiation therapy, and hormone therapy (Bensaleh et al., 2009). Our project uses ultrasound as a mechanism to release drug from alginate microcapsules which serves as our drug carrier.

1.3 MICROCAPSULES AS DRUG CARRIERS:

Microcapsules are generally polymeric hollow spheres that can be filled with drugs, DNA or other biomolecules and can serve as drug delivery vehicles (Fig. 3). Microencapsulation using natural polymers like alginate or chitosan is preferred since it always yields lower toxicity, immunogenicity, good biocompatibility, and it is also biodegradable. Studies show that microcapsules have a selectively permeable outer membrane which allows them to both protect the encapsulated substance and also control the diffusion rate of substances travelling inward and outward (Wang et al., 2006).

![Hollow Microcapsules](image)

Fig. 3 Hollow Microcapsules capable of carrying drugs, DNA, etc.

Using this mechanism drug carrying microcapsules can be directed to elute the drug, once they reach the target site, by using a mechanism to enhance microcapsule membrane
permeability. One such mechanism is the use of therapeutic ultrasound radiation with varying acoustic parameters. Since microcapsules have the ability to remain intact, and be disintegrated only beyond a certain threshold of acoustic intensity, it is a suitable vehicle for triggered drug elution (Shi et al., 2007). Due to these features microcapsules serve as a potential drug carrier for ultrasound-mediated targeted and controlled drug delivery.

1.4 PROJECT PROPOSAL:

Our project requires preparation of drug loaded alginate microcapsules constructed such that they carry both the drug and the ultrasound contrast agents (UCAs). Ultrasound pulses can then cause sonoporation of the capsule membrane leading to drug elution. This procedure can be used for controlled and targeted drug delivery in breast cancer therapy by injecting the loaded microcapsules within the breast tissue at the site of the tumor. Applying therapeutic ultrasound in the breast would then lead to localized drug delivery at the site of the tumor, thus minimizing the side effects of the drug while improving its efficacy.

Our proposal is to incorporate ultrasound contrast agents called targesons within the microcapsules such that, when subjected to ultrasound, the targesons move toward the boundary of the capsule and cause cavitation of the membrane leading to drug release. This would enable a controlled mechanism of delivering drugs within the body triggered by periodic intervals of therapeutic ultrasound application. The manipulation of ultrasound pulse parameters like frequency, intensity and pulse width can control the rate of drug elution. Our project aims to determine the optimal combination of these ultrasound pulse parameters that can achieve controlled drug release with increased efficacy and minimal side effects.
1.5 PROJECT HYPOTHESIS:

The hypothesis to be investigated is that therapeutic ultrasound (1MHz and 2.25MHz) can be used to modulate drug release from large (300-1000 μm) microcapsules carrying ultrasound contrast agents and drug substance.
CHAPTER 2: LITERATURE REVIEW

2.1 MICROCAPSULES IN DRUG DELIVERY:

Microcapsules are polymer based hollow spheres that have diameters ranging from micrometers to a few millimeters. The concept of microcapsules in medicine was initially introduced by Chang in 1964. Research suggests that microcapsules with therapeutic agents have attained importance due to their use in drug delivery, cell and enzyme binding, biomedicine division and micro reactors (Sasaki et al., 2008; Crotts and Park, 1995). The encapsulation method of forming microcapsules is developing at faster rate since Lim and Sun introduced the process back in 1980 (Wang et al., 2004). Recent advances in microencapsulation procedures have made it possible to load microcapsules with drugs, proteins and in other cases cells. Current and future research focuses on tailoring microcapsules to carry encapsulated substances to specific targets in a secure way (Orive et al., 2003).

Studies show that microcapsules have a selectively permeable outer membrane which allows them to both protect the encapsulated substance and also control the diffusion rate of substances travelling inward and outward (Wang et al., 2006). Further, polymer microcapsules have the ability to remain intact and be disintegrated only beyond a certain threshold of acoustic intensity, making it a suitable vehicle for triggered drug elution (Shi et al., 2007) (ref1). A paper by Wang et al. in 2006 states that microencapsulation using natural polymers like alginate or chitosan is preferred since it always yields low toxicity, good biocompatibility, and low immunogenicity and is biodegradable. These evidences show that use of alginate microcapsules is suitable for use as a drug delivery system.

Experimental results from Desai et al., (2009) showed that the drug release rate from microcapsules is dependent on the concentration of the polymer solution. In case of alginate
microcapsules, the higher the concentration of the alginate solution, the more sustained the drug release. It has also been established that lower concentrations of alginate will result in leaky microcapsules which is not effective for controlled and targeted drug delivery.

2.2 THERAPEUTIC ULTRASOUND IN DRUG DELIVERY:

In the early days, ultrasound was viewed as a relatively inexpensive technique and scientists realized that its use could be extended to several clinical applications. In 2009, Pua and Zhong stated that the early clinical applications of therapeutic ultrasound that were implemented successfully were in shock wave lithotripsy (a technique employed to remove kidney stones), thrombolysis (disintegrating blood clots) and physiotherapy.

Early applications of ultrasound in drug delivery was reported by Fellinger and Schmid, who showed that ultrasound could be used for curing polyarthritis by facilitating efficient drug uptake into infected tissues (Ng and Liu, 2002). Researchers like Mitragotri (1991) showed that ultrasound could be successfully used for administering anesthetic drugs like Lidocaine across the skin. Pua and Zhong, 2009, stated that an important aspect of ultrasound use in drug delivery is to attain location specificity, increased drug uptake and decreased harmful systemic effects.

Researchers have shown that drug delivery to solid tumors in a specific and targeted manner was often inefficient thus resulting in toxic effects of drugs in the surrounding tissues. The use of ultrasound as a potential treatment option for cancers and tumors gained impetus owing to these harmful side effects that resulted from conventional treatment options like chemotherapy, radiation therapy, and hormone therapy (Bensaleh et al., 2009). Initially, drug delivery using ultrasound was accomplished at high intensities which resulted in tissue heating leading to localized tissue damage. To avoid this tissue damage, scientists resorted to the use of low intensity ultrasound with UCAs. UCAs serve to absorb the applied low intensity ultrasound
energy and amplify it by emitting secondary ultrasound waves, thus producing effects comparable to those produced by high intensity ultrasound without causing tissue damage (O'Brian, 2007).

Tachibana (1995) reported the earliest use of UCAs in enhancing the effects of ultrasound mediated thrombolysis. Research carried out in the last few years has shown that UCAs can also be used to deliver therapeutic drugs contained within them (O'Brian, 2007). These UCAs can be tagged to reach a specific target site (tumor) and deliver the drug contained within them. The ultrasound energy also increases the target cells' permeability that will lead to enhanced drug uptake (Liu and Nakamura, 2006). "Therapeutic ultrasound has the primary advantage respect to other modalities to be able to penetrate deep into the body and deliver to a specific site thermal or mechanical energy with millimeter accuracy and without damaging the intervening tissue" (Curra and Crum, 2003). This makes it a suitable modality for our targeted drug delivery procedure.

In 2008, Frenkel stated that therapeutic ultrasound is capable of producing bio-effects by two mechanisms – thermal and non-thermal. The thermal mechanisms of therapy are commonly associated with hyperthermia (increase in tissue temperature) due to excessive energy and heat (Bohmer et. al., 2009; Ferrara, 2008). The non-thermal mechanisms of therapeutic ultrasound include cavitation, radiation and micro streaming (Frenkel, 2008; Ferrara, 2008).

Frenkel (2008) stated that cavitation is the most important non-thermal mechanism that is capable of producing biological effects and enhancing drug delivery. Cavitation is a phenomenon occurring in the presence of ultrasound energy that leads to the formation, growth, oscillation and collapse of small gas bubbles (Ferrara, 2008). These gas bubbles can either vibrate or oscillate and result in the increase in membrane permeability of the drug carrier that will lead to
drug release. In a 2003 study, Curra and Crum showed that cavitation has lower effects at low ultrasound frequencies, and therefore operating frequencies for drug delivery applications can span from lower kHz ranges to MHz ranges. Common ultrasound frequencies for such effects are reported to be in the 0.5 – 10 MHz region (Ng and Liu, 2002).

Later studies showed that UCA's have the ability to induce cavitation with much lower ultrasound energy for therapeutic applications (Ferrara, 2008). Researchers found that this reduction in energy would reduce unnecessary exposure to ultrasound and other heating effects that can cause tissue damage (Ng and Liu, 2002). According to Lindner and Kaul (2001) stable gas-filled micro bubbles are effective UCAs in drug delivery applications. In 2008, Frenkel stated that cavitation was affected by both the number and availability of stabilized gas containing micro bubbles. He concluded that cavitation had pronounced effects when the micro bubbles (UCAs), surrounded by a relatively large volume of fluid, underwent acoustic changes near a rigid boundary. This can be achieved by encapsulating these UCAs within microcapsules filled with a large volume of the drug.

It has been reported that non-thermal mechanism of ultrasound activity can also increase the cytotoxicity (toxicity to the cell) of hydrophobic drugs like doxorubicin. By increasing the cytotoxicity of these anti-cancer drugs, their efficiency to kill the tumor cells is increased. However, the mechanism had no such impact on the hydrophilic drugs as seen in the table below (Ng and Liu, 2002). According to a 2008 study by Ferrara, a class of anti-cancer drugs, sonosensitizers with ultrasound, can result in increased treatment efficiency than that achieved by the additive effect of both the mechanisms. Use of hydrophobic sonosensitizers with enhanced cytotoxicity due to the presence of ultrasound, can increase the effectiveness of the treatment process.

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<td>Nitrogen mustard (+)</td>
<td>2 MHz continuous wave (CW) ultrasound; 10 W/cm²; temperature: 26–42.5°C</td>
</tr>
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<td>Doxorubicin (+); Daunomycin (?)</td>
<td>1.92 MHz CW ultrasound; 0.66 W/cm²; temperature: 35°C</td>
</tr>
<tr>
<td>Doxorubicin (+); Amphotericin B (+); Cisplatin (-); Etoposide VP16 (?)</td>
<td>2.025 MHz CW ultrasound; 0.5–2 W/cm²; temperature: 37–43°C</td>
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<tr>
<td>Doxorubicin (+)</td>
<td>2.6 MHz CW ultrasound; 2.3 W/cm²; temperature: 37°C</td>
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<tr>
<td>Doxorubicin (+); Diziquone (+)</td>
<td>1.765 MHz tone burst and pulsed ultrasound; 0.25 W/cm²; temperature: 37°C</td>
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<tr>
<td>Doxorubicin (+)</td>
<td>1.733 MHz CW ultrasound; 0.5–2 W/cm²; temperature: 41–43°C</td>
</tr>
<tr>
<td>Doxorubicin (+); Diziquone (+); Cisplatin (-); Mitomycin C (+)</td>
<td>1.65 MHz tone burst and pulsed ultrasound; 0.5 W/cm²; temperature: 37°C</td>
</tr>
<tr>
<td>Ara-C (+)</td>
<td>48 kHz CW ultrasound; 0.3 W/cm²; temperature: 37°C</td>
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Note: (+): Enhanced activity; (-): no enhancements.

2.3 BIOLOGICAL EFFECTS OF ULTRASOUND ON HUMAN TISSUE:

This section of the literature review concentrates on the biological effects that therapeutic ultrasound (1 MHz to 5 MHz) has on the human tissue. As our project utilizes therapeutic ultrasound for targeted drug delivery, the primary focus of this review is to understand: a) What the safe thresholds of therapeutic ultrasound intensity are, b) What kind of ultrasound beams (pulsed or continuous) are more safe to focus on the human body without adverse side effects like heating or inflammation, c) How long human tissue can be subjected to therapeutic ultrasound exposure without any harm resulting to it and d) What frequency ranges are considered safe by scientists who researched on ultrasound mediated drug delivery using microcapsules.

One of the earliest studies to evaluate the effects of therapeutic ultrasound was carried out in an in-vitro setup by Harvey et al. (O'Brian, 2007). Their experiments subjected bacteria and cells isolated from human tissue to continuous beams of ultrasound waves. The conclusions
made from this study was that exposure of human tissue to therapeutic ultrasound had the potential to disrupt cell membranes. Researchers like O'Brian (2007) have concluded that cell membrane disruptions were due to high exposure levels. Ramirez et al. argued that in-vitro ultrasound experiment results could not be extrapolated to in-vivo conditions since the two conditions were quite different. According to Ramirez et al. the ability of therapeutic ultrasound to disrupt cell membranes is due to its property to cause a phenomenon known as cavitation. He suggested that in-vitro conditions provided an environment that is conducive for cavitation to occur, and was largely responsible for a change in biological characteristics of cell membranes (O'Brian, 2007). Although the human tissue is composed of large percentages of water, these percentages are not large enough to induce the amount of cavitation in-vitro conditions can cause. O'Brian has stated in his research that although cavitation could come into effect when biological tissue is exposed to ultrasound, the number of cells or the amount of tissue affected by cavitation is negligible and unless cavitation effects are widespread across large amounts of tissue there is only a slim possibility that harm could occur.

Another group of researchers (Fahnestock et al.) were experimenting on the effects of therapeutic ultrasound on cadaveric skin extracted from the thigh region. The experimental procedure exposed the skin samples to two types of therapeutic ultrasound beams; pulsed and continuous. A frequency range of 1MHz to 3 MHz and intensity levels of 0.2 W/cm² to 2 W/cm² were selected by Fahnestock et al. to conduct their tests. The conclusion made by them based on their results was that when identical frequencies, intensities and durations of exposure were used, continuous therapeutic ultrasound exposure had more noticeable effects like detachment between dermal and epidermal layers. According to Fahnestock et al. the histological changes occurring in the skin were noticeable under microscopes even under low magnifications. However, an
important aspect these studies proved was that these effects were negligible when pulsed ultrasound beams were used at low intensity and with long exposure times (O'Brian, 2007).

Another researcher, Mitragotri (1991) working on ultrasound mediated transdermal drug delivery corroborated the results of Fahnestock by concluding from his studies on human cadaveric skin that continuous application of therapeutic ultrasound on human skin for more than ten minutes at a frequency of 1 MHz and intensity of 5 W/cm² results in heating or thermal effects that caused a raise in tissue temperature and histological changes in the membranes of cells that line the skins' epidermis. These effects were not seen with pulsed ultrasound.

Studies on the human muscle carried out by researchers (Draper et al. 1993) provided results that showed 1 MHz exposure to continuous therapeutic ultrasound for 10 minute duration on an area of 80 cm² caused a temperature increase of 5°C. Intensity of ultrasound used in this case was 1.5 W/cm². Draper et al. also noted in their studies that this increase of 5°C had no long term adverse effects on the muscle tissue and that for any change in the histology of muscle fibers to take place the temperature rise had to be as high as 13°C. Hogan et al. have claimed that pulsed ultrasound did not cause any heating effects on the tissue under exposure, and a 1-3 week of therapeutic ultrasound application every alternate day actually improved blood flow in patients who suffered from varicose veins with no side effects on underlying body tissues, vasculature or glandular activity. The crucial condition that was to be noted from these studies however was that the intensity of ultrasound did not exceed 3 W/cm² and the application of ultrasound was intermittent.

Many researches have studied the thermal effects of ultrasound. Scientists (Henle, 1983; Sapareto and Dewey, 1984; Dewey, 1994; Dewhirst et al., 2003) have stated that the rates of chemical and enzymatic processes that occur in the mammalian cells are correlated with
temperature and the rate of such processes increase with increase in temperature. If ultrasound use causes an increase in temperature from 37°C to 40°C, no significant biophysical effects are seen. If temperatures are increased beyond 43°C and maintained for certain duration of time, then, it leads to cell death via protein denaturation and enzymatic degradation. Consequently researchers (Dickson and Calderwood, 1980), in their studies have stressed that very long duration of ultrasound exposures (4 hours to 500 hours) are required for cell death due to heating at 40°C and that at temperatures below 40°C there are no irreversible side effects seen in mammalian tissue exposed to ultrasound. Lung and Dewey have shown that ultrasound exposure duration of 100 minutes is required for the temperature of breast tissue to increase to 43°C.

In 1988, the American Institute for Ultrasound in Medicine made a statement on ultrasound effects on mammalian tissue. The AIUM stated that "In the low megahertz frequency range there have been no independently confirmed significant biological effects in mammalian tissues exposed in vivo to unfocused ultrasound with intensities below 100 mW/cm², or to focused ultrasound with intensities below 1 W/cm². Furthermore, for exposure times greater than one second and less than 500s for unfocused ultrasound, or 50s for focused ultrasound such effects have not been demonstrated even at higher intensities, when the product of intensity and exposure time are less than 50 joules/cm²" (AIUM, 1993).

A majority of researchers have studied the biophysical effects of ultrasound contrast agents (UCAs) exposed to ultrasound after they have been introduced into the cardiovascular systems (Holt, R.G., Roy, R.A., 2001). UCA's act as absorbers of ultrasound energy and their introduction into the vasculature and consequent exposure to ultrasound has been shown to cause intense cavitation and blood cell death (Holt, R.G., Roy, R.A., 2001). There is currently very limited literature that shows ultrasound effects on UCAs in solid tissue media. Researchers
(Chavrier and Chapelon, 2000; Holt and Roy, 2001) have claimed that the extent of tissue heating if UCAs are present in solid tissue under ultrasound exposure is 3 to 4 times more than when they're absent. However, if sufficiently low intensities of ultrasound are maintained then the effects can be negligible.

2.4 RESULTS FROM LITERATURE REVIEW:

- The literature review on the biological effects of ultrasound has given us an understanding that high ultrasound intensities result in harm to the human tissue, consequently intensity levels that will be used in our project test methods will be in the mW/cm² range which is well below the level of intensity known to cause adverse effects upon exposure to humans. (Henle, 1983; Sapareto and Dewey, 1984; Dewey, 1994; Dewhirst et al., 2003)

- Past studies have demonstrated that long durations of ultrasound application also has the potential to cause bodily harm, our project test methods would make use of ultrasound beams applied over short intervals of time in an intermittent manner.

- Researchers have also pointed out that pulsed ultrasound is more favorable compared to a continuous focused beam of ultrasound with similar intensities and application periods. Consequently we would use pulsed ultrasound in our tests as opposed to a continuous beam planned prior to the literature review. (Draper et al. 1993, O’Brian, 2007)

- Since UCAs act as absorbers of ultrasound energy, embedding UCAs within the microcapsules would provide a more appropriate strategy to cause drug elution from microcapsules. Since the UCAs absorb most of the ultrasound energy, they would also help in preventing any energy dissipation to surrounding tissue (Chavrier and Chapelon, 2000; Holt and Roy, 2001).
CHAPTER 3: MATERIALS AND METHODS

3.1 MATERIALS:

The equipments and materials required for our project are:

- Medium Molecular Weight Sodium Alginate (A2033)
- Blue dextran dye
- Targestar Ultrasound Contrast Agents (Targesons)
- NaCl and CaCl$_2$
- Gelatin
- Syringe pump atomizer
- Ultrasound Transducers: 1 MHz and 2.25 MHz
- Arbitrary Waveform Generator
- RF Power Amplifier
- Oscilloscope
- A UV-VIS Spectrophotometer
- A high resolution microscope

The following test procedures were carried out to test and validate the proposed hypothesis.

3.2 PREPARATION OF ALGINATE MICROCAPSULES:

To prepare control microcapsules containing only blue dextran dye and test alginate microcapsules containing UCAs and blue dextran dye, the following solutions are initially prepared.

1) 0.9% NaCl solution – 9 gm of NaCl/1000 ml Distilled water.
2) 2% Sodium Alginate solution – 2 gm of Sodium Alginate in 100 ml of NaCl
3) Blue Dextran solution – 20 mg/ml of NaCl

4) 1.5% CaCl₂ solution – 15 gm of CaCl₂/1000 ml Distilled water.

The 2% sodium alginate solution was prepared and allowed to stir overnight. This was used as the base solution to prepare 1.5%, 1.1% or 1% microencapsulation solution with the addition of blue dextran solution for the control solution. The composition of this solution can be calculated using the formula

\[ C_1 V_1 = C_2 V_2 \]

where C1 is the concentration of the base solution

V1 is the volume of the base solution

C2 is the concentration of the microencapsulation solution

V2 is the volume of the microencapsulation solution

In case of the test solution, 0.12 ml of targesons was added per 5 ml of the microencapsulation solution. The volume of blue dextran is adjusted to accommodate for the addition of the targesons. The solution is allowed to stir for 4 hours, sonicated for 10 minutes and then atomized using a syringe pump to yield both control and test microcapsules. The Microencapsulation conditions used for preparing them are shown in the table below:

<table>
<thead>
<tr>
<th># Run</th>
<th>Alginate Concentration</th>
<th>Liquid flow rate (FL)</th>
<th>Air Flow Rate (FA)</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1%</td>
<td>0.5 ml/min</td>
<td>15% - 18%</td>
<td>1000 - 1100μm</td>
</tr>
<tr>
<td>2</td>
<td>1.1%</td>
<td>0.1 ml/min</td>
<td>25% – 29%</td>
<td>200 - 300 μm</td>
</tr>
<tr>
<td>3</td>
<td>1.1%</td>
<td>0.5 ml/min</td>
<td>14% - 18%</td>
<td>1000 - 1100 μm</td>
</tr>
<tr>
<td>4</td>
<td>1.1%</td>
<td>0.5 ml/min</td>
<td>18% - 22%</td>
<td>400 - 500 μm</td>
</tr>
</tbody>
</table>
The experimental setup and the microcapsules generated are seen in the figure below.

Fig. 4 Experimental setup for atomization showing the syringe pump and the microcapsules

The microcapsules formed were isolated, washed 3 times with NaCl. They were then imaged to check their size distribution.

Fig. 5 Microcapsules images as seen under a microscope

3.3 PREPARATION OF GELATIN PHANTOMS:

Gelatin phantoms were used to mimic the breast tissue. The prepared microcapsules were embedded in gelatin phantoms and then subjected to sonication. To prepare these gelatin phantoms, DI water was heated to about 60 °C. Gelatin was added to this at a concentration of 10% by weight to water. The solution was stirred while heating until a clear gelatin solution was obtained. The bottom of the petridish mold was sprayed with mold release and coated with a consistent layer of gelatin measuring about 3 mm in thickness. At a point when the gelatin starts
to set, place the control microcapsules in the center of the mold such that the capsules are
embedded on the gelatin surface. Repeat the procedure for test microcapsules. The phantoms
were then covered and cooled in the refrigerator.

3.4 SOLID MEDIA STUDIES:

To closely represent in vivo conditions where the microcapsules will be embedded in the
breast tissue, we use solid media studies. In these experiments, gelatin or other similar media can
be used to mimic the tissue.

Test Method: This test involves preparation of gelatin phantoms containing the microcapsules
filled with UCAs and blue dextran. The purpose of this test was to determine if the ultrasound
energy has any effect on elution of the dye from microcapsules that are embedded in a solid
media like gelatin. The key parameters of ultrasound that would come into play for this
phenomenon are beam intensity, frequency, pulse width, and application time. The gelatin
phantoms that were prepared earlier were exposed to ultrasound energy and examined under
microscope for microcapsule membrane structural changes. Furthermore, elution studies were
also done on the supernatant to detect the influence of the solid media in the elution of the dye.

Experimental Setup: The sonicator or the ultrasound transducer is connected to an arbitrary
waveform generator, a power amplifier and an oscilloscope as seen in figure 4. The phantom was
submerged in DI water and is exposed to the ultrasound beam from the transducer. The
ultrasound beam used varied in characteristics such as intensity (I), pulse width (W) and
frequency (f) of which several different combinations were used for application on the phantoms
containing the microcapsules.
3.5 MICROSCOPIC EXAMINATION:

The microscopic examination was done to determine any structural changes in microcapsule membrane. Once the application of ultrasound is done, the gelatin phantoms containing microcapsules were subjected to microscopic analysis. This was done to assess any structural changes in microcapsule membrane. This was done to determine if a particular combination of ultrasound parameters is capable of producing physical changes to the membrane that would contribute to drug release.
CHAPTER 4: ECONOMIC JUSTIFICATION

4.1 EXECUTIVE SUMMARY:

Over the years, the development of alternative modes of drug delivery to cure breast cancer has been a subject of interest. Our company, BioCure Ltd. aims at developing a potential non-invasive treatment method to successfully treat breast cancer without any harmful side effects. Breast cancer is widespread in the United States. Among the various types of cancer, breast cancer was estimated to be the single largest type of cancer that affected the female population in 2009. Studies show that among the women population, one in three women will develop cancer over her lifetime, and one in eight women is bound to develop breast cancer.

Chemotherapy is the most common treatment option for breast cancer. It is also a form of adjuvant therapy for breast surgery candidates in order to prevent recurrence of secondary tumors. However, chemotherapy drugs come with a wide range of systemic effects and can affect all rapidly proliferating cells of the body. Although the field of cancer drug delivery is constantly expanding, the toxicity of the anti-cancer drugs makes it important to device a drug delivery mechanism that will minimize the exposure of the drugs to the non-cancerous cells of the body. This mechanism will help in reducing the side effects associated with the drugs and will also enhance the efficacy of the drug at its point of action, the breast tumor. Another requirement is to control the rate of drug release. This will ensure that the tumor is exposed to a constant therapeutic concentration of the chemotherapeutic drug and will prevent drug wastage or accumulation. Given these conditions, we need a drug delivery mechanism that can be localized and controlled to achieve an optimal treatment for breast cancer while minimizing the systemic exposure of the drugs.
Our approach to address this need is with the use of ultrasound-mediated drug release from drug carrying alginate microcapsules. Our solution will be efficient and non-invasive with minimal trauma to improve the quality of life for the breast cancer survivors.

Due to the widespread nature of the breast cancer throughout the world, our treatment modality will have an extensive market. Currently, the US drug delivery market is valued at $80.2 billion and studies estimate this market to grow annually at a rate of 10% through 2012 [9 ps].

Our initial investment for the company will be a maximum of $890,000. However, the recurring expense to operate BioCure Ltd. reduces to $480,000 per year. By the end of the second year, BioCure Ltd. will start generating profit by licensing our technology to other pharmaceutical companies or by contract manufacturing microcapsules. As the profits increase, BioCure Ltd. will invest in new R&D projects for drug delivery systems. A few years down the line the company might team up with a competitor and expand, or exit the market by selling the technology.

4.2 PROBLEM STATEMENT:

While there are promising treatment options for breast cancer, they come with a wide range of side effects that can cause permanent damages. Administering cytotoxic drugs to treat life threatening diseases like cancer are always accompanied by harmful side effects that include total hair loss, severe damage to healthy tissue surrounding cancerous tissue, mouth & throat pain, anemia, nausea, vomiting, dry skin, bladder irritation and so on (Tiguan, 2007). These problems arise mainly because drugs administered for treating cancer are not targeted which implies that drugs meant to kill cancerous tissue act on the healthy tissue that surrounds cancerous tissue as well. Ultrasound mediated drug delivery using microcapsules can
significantly alleviate such problems because this is a targeted (microcapsules can be placed exactly at the site of the cancer for therapeutic effects) and a time controlled (the microcapsules can be made to elute drugs only at those instances when ultrasound is applied over them) mode of drug delivery. So only cancerous tissue is exposed to the cytotoxic drug and healthy tissue is spared.

Additionally, because conventional drug delivery mechanisms are inefficient (as they act even on those regions of the body that do not need therapy) the user consumes a lot more drug than what is required to treat the diseased condition. Since most drugs are expensive this inefficiency adds to the costs borne by the user who is undergoing treatment for his abnormal health condition.

Overall, drawbacks of conventional drug delivery mechanisms clearly indicate that newer and better modes of drug delivery need to be developed, especially for the treatment of cancer. The challenge for BioCure Ltd. is to design a drug delivery system that can deliver anti-cancer drugs to the breast tumor in a targeted and controlled fashion such that systemic exposure of drug is reduced and the treatment efficacy is improved.

4.3 SOLUTION AND VALUE PROPOSITION:

To eliminate disadvantages associated with conventional treatment options, BioCure Ltd. proposes to inject the drug-carrying microcapsules directly at the tumor site. Since our project is based on encapsulating drugs in biocompatible/biodegradable polymer microcapsules; this approach prevents degradation of the drug contained within the capsule by the harsh environments of the human body, the placement of these microcapsules within the tissue inflicted with cancer/tumor and subsequent ultrasound exposure for drug elution facilitates time controlled, targeted drug delivery. Further, we use large microcapsules that are not capable of
entering the smaller blood vessels and hence stay at the target site. On application of therapeutic ultrasound, which is a non-ionizing energy, the UCAs within the capsules effect drug delivery by sonoporation, thus killing the malignant cells at the tumor site without causing any systemic damages. This mode of drug delivery is highly capable of overcoming the drawbacks and problems associated with conventional drug delivery.

The use of ultrasound-mediated drug delivery will minimize side effects of the drug in the surrounding tissues, thus reducing hospitalization time, adjuvant therapies and also post-operative costs. This treatment method would also reduce the trauma associated with current treatment modalities. Further, the raw materials required for this treatment procedure like alginate are simple and readily available, making the technology cost effective and accessible. Our technology can be further extended to develop a localized cure for other types of cancers and also other diseases. Hence, the project would also have significant commercial value if test results obtained look promising. Thus, in addition to having a highly beneficial impact in the clinical drug delivery process, there is substantial probability that the practical development of this project in the future can generate tremendous economic success.

4.4 MARKET SIZE:

In the broad scheme of things, market size boils down to the amount of money involved in producing and consuming a particular "product." In our case this "product" is a unique mode of drug delivery. The size of the drug delivery market is USD 80 Billion with a projected growth rate of 10% annually until 2015. The pie-chart below shows how much money a few top companies are investing just in R&D for alternative means of drug delivery for anti-cancer drugs.
Table 3 below shows the worldwide (United States, Canada, EU and Asia) market size for alternative drug delivering modalities over the last few years.

[www.forbes.com/pharmatech/drug/html]


<table>
<thead>
<tr>
<th>Year</th>
<th>Worldwide Market Size (USD Billions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>43.3</td>
</tr>
<tr>
<td>2005</td>
<td>49.4</td>
</tr>
<tr>
<td>2007</td>
<td>55</td>
</tr>
<tr>
<td>2009</td>
<td>66</td>
</tr>
<tr>
<td>2010</td>
<td>80</td>
</tr>
</tbody>
</table>

4.5 COMPETITORS:

Although various pharmaceutical companies such as BioMarin, MicroCaps®, DuraSolv® and OralSolv® are developing ways to treat diseases with drug filled microcapsules for non-cancer health conditions, there is not much data or market statistic on what companies are focusing on treating cancer with such a technology. It is not publicly known if any pharmaceutical companies are commercializing the delivery of "anti-cancer" drugs through
ultrasound driven mechanisms using microcapsules either. Although it is clear from literature review that Pfizer®, Novartis® and Roche® are researching on anti-cancer drug delivery using microbubbles. So the aforementioned companies would be our direct competitors. In theory however, every pharmaceutical company that produces anti-cancer medication meant for administering in conventional ways could also be viewed as our competitor.

4.6 CUSTOMERS:

Our customers could be large pharmaceutical conglomerates or small startups. Essentially, any company that manufacturers anti-cancer drugs or any company that seeks to deploy their drugs in microcapsules is our potential customer. In fact the competitors described in the preceding sections of this report could turn out to be our biggest customers. The table 4 below lists potential customers and their investment in innovative drug delivering technology.

<table>
<thead>
<tr>
<th>Drug/Pharmaceutical/Biotech Company</th>
<th>Investment in Drug Delivery Technology (In USD Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioVale®</td>
<td>234</td>
</tr>
<tr>
<td>ALZA (A J&amp;J Company)®</td>
<td>1170</td>
</tr>
<tr>
<td>Pzier®</td>
<td>980</td>
</tr>
<tr>
<td>Novartis®</td>
<td>457</td>
</tr>
<tr>
<td>Biomarin®</td>
<td>28</td>
</tr>
<tr>
<td>Skyepharma</td>
<td>112</td>
</tr>
</tbody>
</table>

4.7 COST:

The project comprises of different aspects and the total operating cost for the technology would include the costs associated with each of these individual aspects. When the technology
has been successfully commercialized and approved for clinical use, the factors that would contribute to the operating costs are:

- Microcapsule preparation
- Anti-Cancer drugs
- Ultrasound contrast agents
- Therapeutic Ultrasound beam generating devices
- Other resources- electricity, personnel

The cost for the preparation of microcapsules for ten doses is about $2000 (based on our laboratory usage) inclusive of inorganic salts (sodium chloride and calcium chloride), alginate, anti-cancer drug and UCAs. The estimated costs for the sonication process would range from about $3000 - $7000 considering the use of transducers, oscilloscopes and waveform generators. However, since this is a one time cost, it would not affect the per-patient treatment costs in the long run. Miscellaneous expenses covering the electricity and personnel costs to run the therapeutic ultrasound setup would be a part of the hospitalization costs.

4.8 PRICE POINT:

For a startup company like BioCure Ltd., it is important that we obtain a large customer base and be successful in attracting customers. Pricing our services is going to be a crucial aspect that decides how many customers approach us. Initially our services will be priced low, with the sole motive of attracting customers, both small startups and large pharmaceutical giants. The pricing will be decided based on several parameters. 1) Supply & Demand graph, 2) Market surveys, 3) Expenditure incurred, and 4) Based on the development schedule of the project. We have decided that our licensing price initially will be USD 100,000. Biocure Ltd can also apply for
patents in other countries and obtain approval for marketing and sale overseas. Pricing can be decided based on the currency value of the respective country. An amount equal in value to USD 100,000 will be charged.

4.9 SWOT ASSESSMENT:

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Weakness</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Alternative anti-cancer treatments are</td>
<td>• Part of a highly regulated medical</td>
</tr>
<tr>
<td>the need of the hour - our technology</td>
<td>device/pharmaceutical industry.</td>
</tr>
<tr>
<td>could fulfill that need.</td>
<td>• High initial capital requirements.</td>
</tr>
<tr>
<td>• Enormous potential to benefit millions</td>
<td></td>
</tr>
<tr>
<td>of patients worldwide.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Opportunities</th>
<th>Threats</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Large market with very few competitors.</td>
<td>• Conventional anti-cancer treatment</td>
</tr>
<tr>
<td>• Innovative technology for drug delivery.</td>
<td>options are well established and cost</td>
</tr>
<tr>
<td>Could be extended to treatment of other</td>
<td>effective.</td>
</tr>
<tr>
<td>health conditions e.g. Diabetes.</td>
<td>• Long-term health hazards of using microcapsules for treating cancer is not evident.</td>
</tr>
</tbody>
</table>

4.10 INVESTMENT CAPITAL REQUIREMENTS:

As an initial investment the company would need a capital of $890,000. The break-up of the expenses that are likely to incur are given in Table 5 shown below.
4.11 PERSONNEL:

This company would need personnel with backgrounds in biomedical engineering, pharmaceutical sciences and biotechnology along with people who can oversee the clinical trial and submission for the FDA approval process. We also want to keep our operating costs down so the company would establish with just five personnel. This includes three engineers with expertise in biomedical engineering, pharmaceutical science and biotechnology respectively. One of these engineers could be the president/CEO of the company. We also need one individual with an MBA degree specializing in marketing. Another individual who is well versed with clinical trials and FDA approval process will also be hired.

### Table 5. Initial Capital Requirements

<table>
<thead>
<tr>
<th>Initial Material and Equipment expenses:</th>
<th>$120,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>This includes microencapsulation, Sonication equipments and materials</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial software expenses:</th>
<th>$50,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>This includes results analysis software like MATLAB, Lab View</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FDA Approval Process</th>
<th>$30,000</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Patents</th>
<th>$40,000-60,000</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Clinical Trials</th>
<th>$150,000</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Recurring Fixed Expense Per Month:</th>
<th>$40,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>This includes manpower, utilities, rent</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum Total</th>
<th>$890,000</th>
</tr>
</thead>
</table>

($480,000/year)
4.12 BUSINESS AND REVENUE MODEL:

As a small company, it is imperative that we have attractive business models. Two strategies that the company will deploy are:

1. Licensing our technology to pharmaceutical companies, and

1. Licensing our Technology to Pharmaceutical Companies:

In this mode of revenue generation, we will allow other pharmaceutical companies to make use of our technology on an annual basis for a fee. Yearly license fees of $100,000 would be charged from any company that decides to make use of microcapsulation for drug delivery. An extra cost of $25,000 will be charged for training personnel at the customer site on preparing microcapsules and using appropriate ultrasound frequencies for drug delivery. Companies can encapsulate their own drug and sell them as per market demand. Our company would receive a 10% commission on every batch of microcapsule drug the customer company sells.

2. Contract Manufacturing:

In this model, our company would take charge of the microencapsulation process. A pharmaceutical company that has an interest of administering drugs using our technology but does not want to go through the additional effort to make their own capsules can make use of this revenue generation model. The fixed price that would be charged is $80,000 for per one million capsules. The customer would specify what size of capsules they need and what quantity of drug they would want inside the capsule. Based on the customer's requirements our company will manufacture batches of microcapsule encapsulated drugs. This will benefit companies as they
4.13 PROFIT AND LOSS

The profit and loss projections of the company are given in the table below. This table lists numbers based on 5 years. The break-even curve is generated using this data. The point at which the costs just equal the revenue of the company is termed break-even point. The first year will be spent in developing our technology for commercial use. This would involve carrying out clinical trials with promising results and the entire FDA approval process. We project that one pharmaceutical company approaches us for licensing every month in the second year of our establishment. With the rate of $100,000 per license, at the end of year two we have $1,200,000 in revenue. We also predict that one company would approach us for a contract each month in year two. A minimum of 1,000,000 microcapsule batch contracts are taken. With each contract costing $80,000 to the customer translating into $960,000 from contracts alone. The combined revenue at end of year two from licensing and contracts is $2,160,000. We calculate the return on investment, abbreviated ROI based on the formula given below. Shown in Table 6.

\[
ROI(\%) = \frac{(Total\ Revenue - Total\ Cost)}{Total\ Cost} \times 100
\]

Table 6. ROI Calculation over the years

<table>
<thead>
<tr>
<th>Year</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost (USD)</td>
<td>890,000</td>
<td>480,000</td>
<td>480,000</td>
</tr>
<tr>
<td>Revenue (USD)</td>
<td>0</td>
<td>2,160,000</td>
<td>2,160,000</td>
</tr>
<tr>
<td>Profit/Loss (USD)</td>
<td>-890,000</td>
<td>1,680,000</td>
<td>1680,000</td>
</tr>
<tr>
<td>ROI (USD)</td>
<td>-100%</td>
<td>350%</td>
<td>350%</td>
</tr>
</tbody>
</table>

Comment [LPW1]: Please add a P&L graph in addition to your break even plot.
The chart below shows us the break-even point, which turns out to be the 19th month since our establishment. Basically, from the 19th month (i.e. month 7 of year two) the company will start making profits.

Fig. 9 Break-even Analysis

Fig. 10 Profit and Loss Graph
It is also imperative that we assess the flow of financials within the company. This can be achieved by using mathematical tools like the Nordon-Rayleigh (N-R) curve. A control over the costs and budget of the company can then be obtained. Figures below show the funding profile over the first year of the company's establishment and the cumulative funding over time. The table 7 given below shows the cumulative cost driver calculation.

Table 7. Cumulative Cost Drivers

<table>
<thead>
<tr>
<th>Technical risk</th>
<th>Personnel Expertise</th>
<th>Advantage based on innovative technology</th>
<th>Normalized cost driver</th>
</tr>
</thead>
<tbody>
<tr>
<td>A= 0.005</td>
<td>A=0.002</td>
<td>A=0.001</td>
<td>A=0.03</td>
</tr>
</tbody>
</table>

Fig. 10 Funding profile for the first year
Figure shows the funding profile for the first year.

![Cumulative Funding for the First Year](image)

Fig. 11 Cumulative funding over the first year

Figure shows cumulative funding over the first year

4.14 EXIT STRATEGY:

We see ourselves as a small startup company with immense potential to grow big with our innovative technology. As is the trend with similar companies in the medical device/pharmaceutical industries, if we grow to become highly profitable and pay off our investors, we would continue to be an independently owned private company. If there is an opportunity to sell out to a very large pharmaceutical conglomerate making a lucrative offer, then it might be an option we would seriously consider. If the company is in the need of large amount of capital for investing in new technology then we might even consider going public via an IPO.
CHAPTER 5: PROJECT SCHEDULE

Based on the schedule of our activities the important milestones of our project identified were the:

- Selection of Topic
- Literature Review
- Analysis of Experimental Results
- Optimization of Results and
- Preparation of the Final Project Report
All the tasks have been completed and are represented in blue based on their duration on the Gantt chart.

Fig. 13 Project Gantt Chart
CHAPTER 6: RESULTS AND DISCUSSION:

We successfully prepared and imaged acoustically sensitive microcapsules (ASMs) encapsulating ultrasound contrast agents and blue dextran dye (Model-drug) over a wide range of sizes (300 – 1000 μm) for achieving the purpose of intra-tumoral injection or implantation. We clearly see UCAs within the ASM at high magnification.

**Sonication Parameters:**

The sonication experiments were carried out using 1MHz SF transducer with focal length of 2 inches. The results were analyzed by doing microscopic examination of the microcapsules before and after sonication. The sonication conditions used were as follows:

1) Wavelength – sine wave, continuous  
2) Amplitude – 70 mV peak to peak  
3) Transducer – 1MHz, Spherical Focus, 2”  
4) Size of microcapsules: 300μm, 400-500 μm, 1000 -1100 μm  
5) Time of sonication: 10sec, 30sec, 40sec and 60sec  

Following on next few pages are the images of the microcapsules obtained under different magnification. These images also contain control batches without UCAs and beads of 1.1% and 1% alginate concentrations.
Fig. 14 Different size microcapsules with UCAs and blue dextran dye prepared by atomization.

**Sonication of 1% Alginate Control microcapsules and Test MCs (1000µm – 1100µm):**

<table>
<thead>
<tr>
<th>Before Sonication</th>
<th>Sonicated (30sec, 10X)</th>
<th>Sonicated (60sec, 10X)</th>
</tr>
</thead>
</table>

Ultrasound Contrast Agents

Microcapsule with UCA & BD (20X, 40X magnifications)

Small MCs (300- 350 µm)  Medium MCs (400- 500 µm)  Large MCs (1000 –1100 µm)
These microcapsules were prepared from 1% alginate solution containing blue dextran and UCAs in test samples as well as Control batch without UCAs. The Microencapsulation conditions used were followed from table 2 mentioned above. The microscopic examination revealed better results while the control microcapsule images were confusing because it showed the effect like the test samples with UCAs. The test sample sonicated for 30sec showed bubbles effect while the sample that was sonicated for 60sec showed burnt effect. Further experimentation was carried out by increasing alginate concentration to 1.1% and decreasing the microcapsule size range from 1000 μm to 500 μm.

**Sonication of 1.1% Alginate Microcapsules with UCAs and Blue Dextran:**

Following are the images of 1.1% Alginate microcapsules with UCAs of size range 400 - 500μm, which are sonicated for 40seconds and imaged under 10X and 20 X magnifications.

*Set 1*

Control Before sonication (10X)  Control Sonicated (30sec, 10X)  

Fig. 15 Pre and Post- sonication images of control and test MCs with UCAs in Gelatin phantoms using a Light Microscope
Fig. 16 Post sonication images of microcapsules with UCAs

The microscopic observation of 400 – 500 μm size microcapsules was done on the gelatin phantom after sonication and revealed reproducible results. Only test samples were prepared for sonication experiment in total of five sets and were sonicated for 40 seconds. Set 1 had structural change in the microcapsule membrane, Set 2 showed charring effect, Set 3 had bubbling effect while Set 4 and Set 5 had structural change in microcapsule membrane.
Fig. 17 Microcapsule (500µm) with UCAs Sonicated with 2.25MHz, CW for 60sec in gelatin

Sonication was done with 2 different transducers: 1MHz and 2.25 MHz and both had different focal widths, depths, and extents (2.25 (1.25" focus) or 1 MHz (2" focus) - focal extent at 90% intensity = 1.5 or 5 mm, -6dB beamwidth= 1.0 or 4.2 mm). 2.25MHz required more exact optical acoustic alignment but produced more pronounced changes. 1 MHz transducer produced more subtle changes with more freedom for alignment. Upon sonication with CW ultrasound, definite visual changes were noted in the membrane, primarily with those capsules that had UCAs. Controls without UCAs did not experience rupture under similar conditions. Mechanical measurements revealed that stiffness of 1% alginate beads (used for sonication studies) could be on the order of several KPa requiring the use of CW ultrasound for rupture. Permeable capsules measured had significantly lower moduli than the beads as expected.

Future experiment was carried out using pulsed sequence of ultrasound at different duty cycles as 0.1%, 1%, 10%, 30% and 100% under two different amplitudes, 60mVpp and 90mVpp. The size of the microcapsules was also decreased to 300 µm. Test samples for each duty cycle and two control samples without UCAs were prepared. The control samples were sonicated for 30sec at 10% duty cycle and at both amplitudes 60mVpp as well as 90mVpp. The time of
exposure for gelatin phantoms containing capsules was constant at 30 seconds. Sonication was done with 1MHz Spherical Focus transducer with 2” focus. Following are the results that were generated through microscopic examination under 20X magnification.

For 60mVpp over 0.1%, 1%, 10%, 30% and 100% duty cycle using 1MHz SF transducer, cavitation effect was seen for short duty cycle while target release was observed in 10% duty cycle for 300 -400 µm size microcapsules.
Following are the images of sonicated microcapsules in gelatin at 90mVpp amplitude. The size of microcapsules was 300 - 400 µm and all the samples including control was sonicated for 30 seconds.

For 90mVpp over 0.1%, 1%, 30% and 100% duty cycles using 1MHz Spherical focus transducer, cavitation bubble formation was seen for shorter duty cycles and targets release.
effect was seen for 100% duty cycle. The burning as well as cavitation effect was seen as we proceed from shorter duty cycles to longer duty cycles.

CHAPTER 7: CONCLUSION

Microscopic analysis of pre and post sonicated microcapsules showed pronounced effect. Bubbling effect was seen in post sonicated microcapsules with 30 seconds of exposure time while charring effect was observed in post sonicated microcapsules with 60 seconds of exposure time. Further experimentation was carried out with 1.1% alginate microcapsules' of 400 – 500 μm size range, and was subjected for 40 seconds to ultrasound. A change in microcapsule membrane is seen and some microcapsules have pronounced ultrasound effect when the number of capsules were decreased in the gelatin phantoms and were placed exactly under the transducer focus. 10s of continuous wave ultrasound caused membrane damage in microcapsules that encapsulated both UCAs and blue dextran dye (Model- drug), while control capsules with no UCAs did not show any results under similar conditions indicating that ultrasound sensitivity is specifically enhanced with presence of UCAs. The pulse sequence experiment which was carried out at two different amplitudes for smaller size microcapsules of about 300 μm and were sonicated for 30 seconds at five different pulsing sequence (0.1, 1, 10, 30 and 100% duty cycle) showed pronounced effect. At 60 mVpp the cavitation bubble formation effect was more pronounced while at 90 mVpp burning or heating effect was more pronounced.

In the future, several quantitative studies with fluorescence imaging are planned to assess drug release from microcapsules with both continuous wave and pulsed ultrasound.
REFERENCES:


Publication by ACS - Date accessed 04/27/2010


http://www.syringepump.com/NE-1000.htm (Date accessed - 04/28/2010)


http://www.sigmaaldrich.com/catalog/ProductDetail (Date accessed - 04/28/2010)

http://www.invivoimagingssolutions.com/Targeson (Date accessed - 04/28/2010)