

# Sonoporation: Therapeutic Ultrasonic Waves

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## ABSTRACT

Ultrasound, traditionally a diagnostic modality, is emerging as a very effective and promising physical method for delivering drugs, nucleic acids and for gene therapy. Sonoporation is a technique based on ultrasonic waves that employs the acoustic cavitation of microbubbles that generates transient, non-specific pores on membranes to enhance delivery of large molecules such as DNA into viable cells for potential targeted drug delivery and non-viral gene transfection. This transient pores after ultrasound exposure allow permeation to extracellular molecules for a limited time window into the interior of cells which are non-permeable. Sonoporation is used in the delivery of therapeutic agents including genetic material, proteins, monoclonal antibodies and chemotherapeutic agents. It can serve as a novel application for treatment of oral squamous cell carcinoma because of its non-invasiveness and potential to treat deeper tissues in the body.

**KEYWORDS:** Sonophoresis, Cavitation, Microbubble, Gene Therapy

## INTRODUCTION

As an established therapeutic method, ultrasound (ULTS) is used for bone fracture healing, hyperthermia and the ablation of solid tumors. Furthermore, in this newly emerging field, ULTS mediated microbubble destruction, a noninvasive approach, has been shown to possess significant potential to increase the permeability of cell membranes and tissues to various substances. Since ULTS mediated microbubble destruction is able to reversibly disrupt biological barriers, particularly cell membranes, large quantities of molecules may then be delivered into tumor cells, particularly drug resistant cells. The mechanism by which this occurs is considered to be sonoporation, resulting from oscillations of the gas bubbles in the media, which cause cavitation close to the cell surface and subsequent membrane disruption that allows increased drug internalization. It has been demonstrated that intracellular uptake is greatly enhanced by diagnostic microbubbles used for ULTS imaging. At particular ultrasonic frequencies, microbubbles have been shown to greatly enhance transient sonoporation. These microbubbles, oscillating in the presence of ULTS, create localized shear stress or 'microstreaming' or they may expand and collapse ('transient cavitation') to create intense local heating and pressure.<sup>1</sup> Schlicher *et al.* demonstrated transient pores (<28 nm diameter) in the plasma membrane of cells, following exposure to low-frequency ULTS (24 kHz).<sup>2</sup> "When sound is emitted at a particular frequency, the sound waves disrupt the lipid bilayers," said Mitragotri. He pointed out that the ideal ultrasound frequency range for the transdermal delivery of biologics is 50-60 KHz. "The higher the frequency, the more dispersed the transmission".<sup>3</sup>

## SONOPORATION

Sonoporation is defined as the interaction of ultrasound with ultrasonic contrast agents to temporarily permeabi-

lize the cell membrane allowing for the uptake of various substances such as DNA, drugs, and other therapeutic compounds, from the extracellular environment.<sup>4</sup> Sonoporation is used in the delivery of therapeutic agents including genetic material, proteins and chemotherapeutic agents into the cell, in a cell disruption process called transfection or transformation. It employs the acoustic cavitation of microbubbles to enhance delivery of these large molecules. This technique is similar to, and in some cases found superior to, electroporation. Thus, sonoporation is a promising drug delivery and gene therapy technique, which unlike other methods of transfection or chemotherapy, combines the capability of enhancing gene and drug transfer with the possibility of restricting this effect to the desired area and the desired time.<sup>5</sup>

The first published report on sonophoresis dates back to 1950s. Fellingner and Schmidt reported successful treatment of polyarthritis of the hand's digital joints using hydrocortisone ointment with sonophoresis. It was subsequently shown that hydrocortisone injection combined with ultrasound "massage" yielded better outcome compared to simple hydrocortisone injections for bursitis treatment.<sup>3</sup> Cameron reported success using carbocaine sonophoresis for closed Colle's fractures.<sup>6</sup>

**Principle:** The technique of sonoporation is based on ultrasonic waves. The source of these waves is a piezoelectric crystal transducer made of lead-zirconate-titanium or barium titanate.<sup>4</sup> These waves are produced in response to an electrical impulse in the piezoelectric crystal, allowing the conversion of electrical energy into mechanical or vibrational energy. Following the external perturbation, groups of molecules oscillate in phase and transmit their kinetic energy to nearby molecules.<sup>7</sup>

For sonophoresis delivery, the desired drug is dissolved

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in a solvent and applied on the skin. Ultrasound is applied by contacting the transducer with the skin through a coupling medium to ensure a proper contact between the transducer and the skin. This medium can be the same as the solvent used to dissolve the drug or it can be a commercially available ultrasound coupling gel (for e.g. Aquasonic, Polar, NJ).<sup>3</sup>

## MECHANISM OF ACTION

The ultrasound radiation is given from ultrasound machine to the microparticles suspension. The ultrasound radiation is applied effectively to generate cavitation bubbles, wherein the cavitation bubbles collapse and transfers their energy into the skin area thus causing the formation of pores in the skin area.<sup>4</sup> Thus, sonoporation employs the acoustic cavitation of microbubbles to enhance delivery of these large molecules through the formation of transient pores in the cell membrane facilitating transmembrane transport of drugs into the cell. Inertial cavitation is the process of formation, oscillation and collapse of gaseous bubbles driven by an acoustic field.<sup>8</sup>

The ultrasound frequency may be 20 KHz and the ultrasound intensity may be in the range of 5 W/cm<sup>2</sup> and 55 W/cm<sup>2</sup>. The tip may have a distal end located at a distance from the membrane in the range of 1 millimeter to 10 millimeters. The ultrasound radiation may be continuous or pulsed and it may be applied for a period of time in the range of 30 seconds to 5 minutes, preferably 1 minute for continuous exposure or about 10 to 20 minutes for pulsed exposure with a 5% duty cycle, respectively. The formed pores may have a diameter in the range of 1 micrometer to 100 micrometers.<sup>4</sup>

There are three distinct sets of ultrasound conditions based on frequency range and applications<sup>3</sup>:

- High-frequency or diagnostic ultrasound in clinical imaging (3–10 MHz).
- Medium-frequency or therapeutic ultrasound in physical therapy (0.7–3.0 MHz).
- Low-frequency or power ultrasound for lithotripsy, cataract emulsification, liposuction, cancer therapy, dental descaling and ultrasonic scalpels (18–100 kHz).

Although considerable attention has been given to the investigation of sonophoresis in the past years, its mechanisms were not clearly understood, reflecting the fact that several phenomena may occur in the skin upon ultrasound exposure. These include<sup>9</sup>:

1. Cavitation effects.
2. Thermal effects.
3. Induction of convective transport.
4. Mechanical effects.

**Cavitation effect:** Cavitation is the formation of gaseous cavities in a medium ultrasound exposure. The primary cause of cavitation is ultrasound – induced pressure variation in the medium.<sup>10</sup> Cavitation involves the generation and oscillation of gaseous bubbles in a liquid

medium and their subsequent collapse when such a medium is exposed to a sound wave, which may be an ultrasound. It can generate violent microstreams (fig.1), which increase the bioavailability of the drugs.<sup>3,10</sup> Cavitation occurs due to the nucleation of small gaseous cavities during the negative pressure cycles of ultrasound, followed by the growth of these bubbles throughout subsequent pressure cycles. Whenever small gaseous nuclei already exist in a medium, cavitation takes place preferentially at those nuclei.<sup>3</sup> This cavitation leads to the disordering of the lipid bilayers and formation of aqueous channels in the skin through which drugs can permeate.<sup>11</sup> The minimum ultrasound intensity required for the onset of cavitation, referred to as cavitation threshold, increases rapidly with ultrasound frequency.<sup>3</sup>

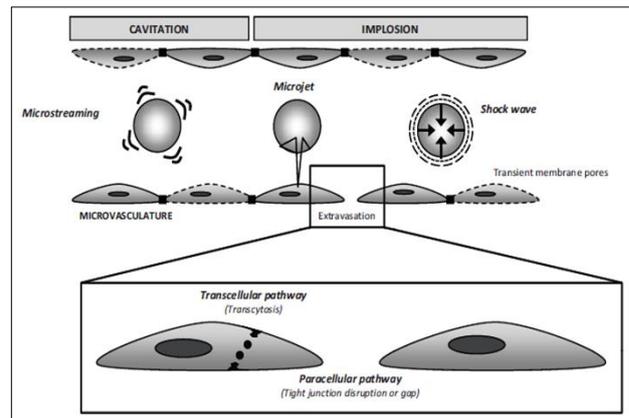


Fig 1 – Cavitation effect

**Thermal effects:** Absorption of ultrasound increases temperature of the medium. Materials that possess higher ultrasound absorption coefficients, such as bone, experience severe thermal effects compared with muscle tissue, which has a lower absorption coefficient. The increase in the temperature of the medium upon ultrasound exposure at a given frequency varies directly with the ultrasound intensity and exposure time. The absorption coefficient of a medium increases directly with ultrasound frequency resulting in temperature increase.

**Convective transport:** Fluid velocities are generated in porous medium exposed to ULTS due to the interference of the incident and reflected ULTS waves in the diffusion cell and oscillations of the cavitation bubbles. Fluid velocities generated in this way may affect transdermal transport by inducing convective transport of the permeant across the skin, especially through hair follicles and sweat ducts.<sup>9</sup>

**Mechanical effects:** At frequencies greater than 1 MHz, the density variations occur so rapidly that a small gaseous nucleus cannot grow, and cavitation effects cease. But other effects due to density variations, such as generation of cyclic stresses because of density changes that ultimately lead to fatigue of the medium, may continue to occur. Lipid bilayers, being self-assembled structures, can easily be disordered by these stresses, which result in an increase in the bilayer permeability.

Thus cavitation induced lipid bilayer disordering is found to be the most important cause for ultrasonic enhancement of transdermal transport.<sup>3</sup>

## ADVANTAGES & DISADVANTAGES

### Advantages of Sonoporation<sup>9</sup>

- Enhanced drug penetration (of selected drugs) over passive transport
- Allows strict control of transdermal penetration rates
- Permits rapid termination of drug delivery through termination of ULTS
- Skin remains intact, therefore low risk of introducing infection
- Less anxiety provoking or painful than injection
- In many cases, greater patient satisfaction
- Not immunologically sensitizing
- Less risk of systemic absorption than injection

### Disadvantages of Sonoporation<sup>9</sup>

- Can be time-consuming to administer.
- Minor tingling, irritation, and burning have been reported (these effects can often be minimized or eradicated with proper ultrasound adjustment).
- Stratum corneum must be intact for effective drug penetration.

## APPLICATIONS

- Gene delivery
  - Osteoinduction
  - Induction of dental pulp stem cell differentiation into odontoblasts
  - Site – specific gene delivery
  - DNA transfer
- Local Drug delivery
- Targeted Drug delivery
- Tumor cell killing
- Induction of Apoptosis

**Gene delivery:** Gene therapy is a technique for correcting defective genes that are responsible for disease development, most commonly by replacing an 'abnormal' disease-causing gene with the 'normal' gene. A carrier molecule (vector) is usually used to deliver the therapeutic gene to the target cell. Topical delivery of the vector–gene complex can be used for target cells within the skin, as well as for the systemic circulation (fig 2). The identification of genes responsible for almost 100 diseases affecting the skin has raised the option of using cutaneous gene therapy as a therapeutic method<sup>3</sup>.

Its applications in the field of oral medicine may act as an eye opener for oral physicians who are not familiar with this life altering technique<sup>4</sup>.

Its applications are as follows-

- Osteoinduction: Bone morphogenetic protein is believed to participate in bone healing and

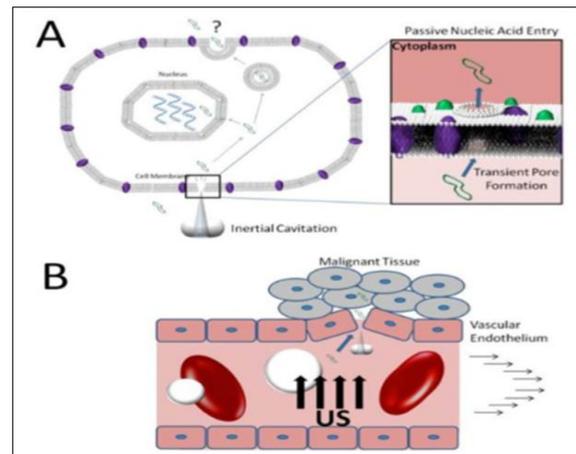


Fig 2 – Gene is delivered to cells, allowing them to produce their own therapeutic proteins.

regeneration. Gene transfer approach is a promising option for utilizing Bone morphogenetic protein.

- Induction of dental pulp stem cell differentiation into odontoblasts: The long-term goal of dental treatment is to preserve teeth and prolong their function. This technique has shown some light in this dream which in future will definitely become possible and will possibly allow the natural complete restoration of teeth<sup>4</sup>.

Misako Nakashima (2003) studied that gene therapy has the potential to induce reparative dentine formation for potential pulp capping. In their research acoustically active materials, microbubbles, have been developed to bind or trap genes. The sonotransfection was performed in the amputated dental pulp. Gene transfer of *Gdf 11* was optimized to induce differentiation of pulp cells into odontoblasts in vitro by sonoporation with microbubbles. Dental pulp tissue irradiated by ultrasound showed a significant efficiency of gene transfer which stimulated the reparative dentin formation during pulpal wound healing in canine teeth (Fig 3). These results provide the scientific basis and rationale for gene therapy in endodontic treatment<sup>12</sup>.

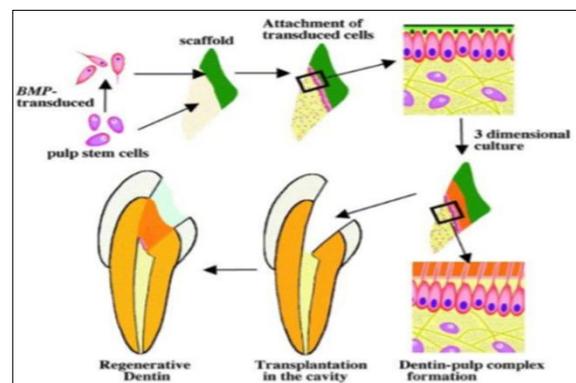


Fig. 3 Induction of dental pulp stem cell differentiation into odontoblasts

- DNA transfer: Delivery of nucleic acid to a target tissue together with a means of facilitating efficient entry of nucleic acid into cell populations of that tissue remain amongst the principle challenges to effective gene therapy in the treatment of a variety of disorders including cancer, cardiovascular disease and inherited immune deficiencies. The development of nonviral gene transfer methods would be a valuable addition to the gene-therapy armamentarium, particularly for localized targeting of specific tissue volume<sup>4</sup>.

**Drug Administration:** Transdermal drug delivery is an attractive alternative to conventional drug delivery methods such as oral administration and injections. Local drug delivery ensures sufficient drug concentration at the diseased site while limiting toxicity for healthy tissues<sup>4</sup>. Drugs that can be delivered transdermally include NSAIDs, anesthetic agents, antibiotics, anti-cancer drugs, fibrinolytic drugs, corticosteroids, insulin and vasodilators. These drugs can be incorporated into microbubbles, which in turn can target a specific disease site using ligands such as the antibody. The drugs can be released ultrasonically from microbubbles that are sufficiently robust to circulate in the blood and retain their cargo of drugs until they enter an insonated volume of tissue (fig 4). The intensity commonly used for transdermal drug delivery is 0.5 – 3.0 W/cm.<sup>13</sup>

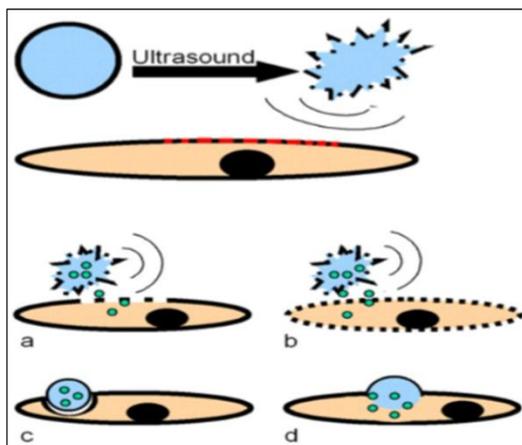


Fig 4 – Drugs can be incorporated into microbubbles, which in turn can target a specific disease site using ligands

There are two strategies for local drug delivery when microbubbles and ultrasound are used. In the first strategy, the microbubbles serve as cavitation nuclei only. Cavitation will cause increased permeability of the cell membranes and disrupt the shell of codelivered carriers, thereby releasing the enclosed drugs, resulting in an increased local drug concentration and an enhanced extravasation of the drug. This approach may be principally successful in the microvasculature. The second strategy of using microbubbles in drug delivery is by loading the microbubbles with the drugs. In this case, the destruction of microbubbles leads to the release of the drug and extravasation of the drug due to the increased number of pores in the vasculature. Microbubble disruption is an insufficient indicator of sonoporation.<sup>14</sup>

The following physical phenomena may facilitate drug delivery:

- They are thought to create holes in the cell membrane, which facilitate entry of drug or plasmid DNA into cells. Shock waves, bubble wall motion in both stable (i.e. bubble expansion without collapse) and inertial cavitation (i.e. bubble expansion and collapse), and microjets may cause the membrane to deform beyond the threshold strain for rupture<sup>15,16</sup>. The influence of cavitation in cell membrane permeabilization was confirmed by experimental and theoretical analyses.<sup>17</sup>
- As a rise in temperature influences the fluidity of phospholipid bilayer membranes, cell membrane permeability could be changed directly as a consequence of the increased bilayer fluidity.<sup>18</sup>
- Endocytosis or phagocytosis, active membrane transport mechanisms, may also be involved in the uptake of the bubble, bubble fragments, or material entrapped in microbubbles.<sup>19</sup>
- Exchange or fusion of the phospholipid microbubble coating with the phospholipid bilayer of a cell membrane could result in delivery of the cargo of the microbubble directly into the cytoplasm of the cell, with the possibility of further uptake in endosomes or delivery to the cell nucleus.
- When mechanical energy within the ultrasound wave is absorbed by protein, it could alter the structural conformation (three-dimensional shapes) of an individual protein or the function of a multimolecular complex. It could induce resonant activity in the protein (frequency response), modulating the function of the multimolecular complex, dislodge an inhibitor molecule, leading to activation of a signal transduction pathway, or inactivate unwanted compounds by breaking up a multimolecular complex resulting in a loss of function or decrease in activity.<sup>20,21</sup>
- The shell of drug delivery systems is disrupted and thereby the enclosed drugs are released.<sup>22</sup>
- In the presence of oscillating bubbles, drug transport into cells is enhanced by orders of magnitude over transport by diffusion alone. Microstreaming can transport drugs at high velocities.

**Tumor Cell Killing:** The Ultrasound-mediated destruction of microbubbles has been proposed as an innovative non-invasive drug delivery system for cancer therapy (fig 5). This will not act only by killing the cells but can give a new favourable look to chemotherapy over other treatment options. The patient will be saved from adverse effects of anticancer drugs which usually act as double edged swords.<sup>4</sup>

Optimization of ULTS parameters for *in-vivo* bleomycin delivery was undertaken by Larkin et al. (2008), and an effective antitumor effect was demonstrated in solid tumors of both murine and human cell lines. Cell death after treatment was shown to occur by an apoptotic mechanism. The results achieved in these experiments were equivalent to those achieved using electroporation

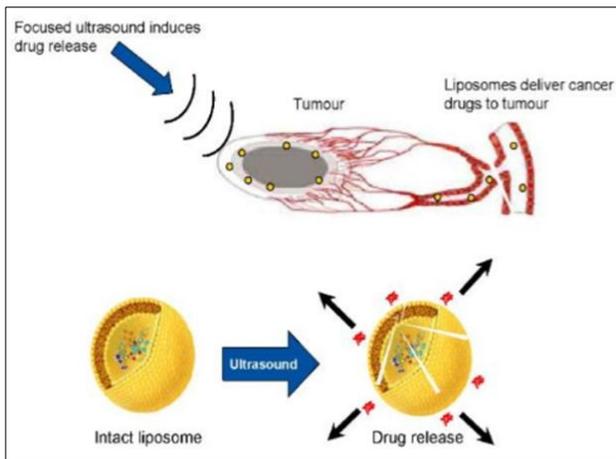


Fig 5 - Tumor Cell Killing

to mediate delivery of bleomycin-electrochemotherapy. They found that, although temperature rises of up to 5 degrees C occur using the optimized ULTS conditions, this effect is not responsible for the potentiated drug cytotoxicity. This technique could be used with focused ULTS or with endoscopic ULTS probes to develop a localized and effective anticancer treatment with little or no systemic toxicity<sup>9</sup>.

**Induction of Apoptosis:** Apoptosis is an organized process of cell death occurring naturally for unneeded cells. Ashush et al.<sup>23</sup>, and Ando et al.<sup>24</sup>, concluded that exposure of cells to ultrasonic cavitation induced apoptosis in addition to the conventionally reported instantaneous cell lysis and necrotic disintegration. This process may be used in future for killing cancerous cells and other cells of benign growth before malignant change take place or reduction in size of the growth before surgeries<sup>13</sup>.

## FUTURE PROSPECTS

The field of sonoporation and ultrasound-enhanced drug/gene delivery has expanded tremendously during the past decade, /and promises to radically change the way in which we inject drugs in the near future.<sup>3,25</sup> As ultrasound mediated transdermal transport via skin patches provides a sustained delivery of the drug over a period of about 7 days, it eliminates the danger posed by the administration of, say, cancer chemotherapeutic agents. These toxic agents can cause even death when given at dosages that are needed to be effective. In the future, drug release systems aided by ultrasound may be able to provide slow release of vaccines such as that for tetanus, which need repeated booster shots; or for an AIDS vaccine. Researchers are currently exploring the applications of low-frequency sonophoresis in various areas like cutaneous vaccination, transdermal heparin delivery, transdermal glucose monitoring, and delivery of acetyl cholinesterase inhibitors for the treatment of Alzheimer's disease, treatment of bone diseases and Peyronie's disease and dermal exposure assessment. The possibilities seem endless.<sup>3</sup>

## CONCLUSION

Sonoporation involves the use of ultrasound with ultrasonic contrast agents to enhance cell permeabilization. With this method it is possible, by using ultrasound and contrast microbubbles, to deliver therapeutic compounds non – invasively into specific target cells. Sonoporation has the potential to deliver drugs and genes to appropriate cells within a patient in a way that is temporally and spatially specific, efficient and safe. Therefore, instead of continuously attempting to create new drugs, sonoporation can target existing therapeutic vectors to the affected part of the body. Also, its application in the field of oral medicine may act as an eye opener for oral physicians who are not familiar with this life threatening technique.

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