Sonoporation-Invigorating Sound in Dentistry: A Review

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Ultrasound (US), traditionally a diagnostic modality, and is emerging as a non-invasive therapy using local drug delivery and gene therapy. US exposure generates bio effects that result in shear stress, tissue heating, and cavitation effects, which are used in therapeutic applications. Sonoporation employs these effects to enhance delivery of large molecules such as DNA into the cells which is applied to muscle, head and neck tumor, in a cell disruption process called transformation and increases the permeability to bioactive materials, which is usually used in molecular biology and gene therapy. Nevertheless, it has recently become popular as a technique to enhance drug release from drug delivery systems. This review presents the main findings in the field of sonoporation, namely drug delivery, gene delivery and DNA transfer and its applications in dentistry.

Keywords: Gene therapy, Sonography in oral diseases, Sonoporation, Ultrasound

INTRODUCTION
Ultrasound (US) is an oscillating sound with a frequency greater than the range that humans can hear.1 US has a broad range of applications in the field of medicine and dentistry.

The applications of US use intensities as shown in Table 1.2

Ultrasonic wave frequencies reach up to a depth of 2-5 cm of tissue.3 The therapeutic effects of US are derived from its thermal and non-thermal properties. Sound waves at high intensity (1-1.5 W/cm²) cause tissue vibration that produces heat in the treatment field.4 At low intensity (<0.3 W/cm²) the non-thermal property of sound waves are utilized in the therapeutic field.5 US application in dentistry includes the treatment of myofacial pain dysfunction syndrome (MPDS), silolithotripsy of salivary calculi, Temporomandibular disorder (TMD), craniofacial deformities, root canal procedure, cleaning of instrument prior to sterilization and dentures.2

SONOPORATION
Definition
Sonoporation or cellular sonication is a procedure in which temporary modification of the permeability of the cell membrane is achieved by the use of US waves which further allows the uptake of certain substances from the extracellular environment.6 This membrane alteration is transient, which leaves the compound trapped inside the cell (Figure 1).

Mechanism of Action of Therapeutic US (TU)
The biophysical effects of TU have been examined mainly in vitro studies.7 Therapeutic results obtained by ultrasonic energy are thought to be due to:4

Table 1: Applications of US based on frequency used

<table>
<thead>
<tr>
<th>Application</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Diagnostic</td>
<td>5 and 500 W/cm</td>
</tr>
<tr>
<td>Surgical</td>
<td>More than 300 W/cm</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>Between 1 and 3 W/cm</td>
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Figure 1: Sonoporator7

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which induces differentiation of pulp stem cells into odontoblasts.14

Local Drug Administration
A required amount of drug concentration at the diseased site is achieved by local drug delivery while limiting toxicity for healthy tissues. Transdermally delivered drugs includes antibiotics, anesthetic agents, vasodilators, fibrinolytic drugs, anti-cancer drugs, corticosteroids, insulin and non-steroidal anti-inflammatory drugs.15 Ultrasonically the drugs are released from microbubbles that circulate in the blood at required concentration until they enter an insonated volume of tissue (Figure 2).

Advantages:16
1. Allowed strict control of transdermal penetration rates
2. Less anxiety is provoking and less painful then injections
3. It also allows greater patient satisfaction
4. Less systemic absorption
5. The drugs given were less immunological sensitizing.

Tumor Cell Killing
A non-invasive drug delivery system has been proposed for cancer therapy, by US-mediated destruction of microbubbles.14

The Iwanga et al.17 in 2007 in Japan studied the efficiency of sonoporation toward growth inhibition of human gingival squamous carcinoma cells in vitro and in vivo. The Ca9-22, a human gingival squamous carcinoma cell line was used in this study. Sonoporation was used to deliver bleomycin and transfec a cdtB-expressing plasmid into Ca9-22 cells in vitro and in vivo. The results showed that tumors nearly disappeared in Ca9-22 cell-implanted treated with bleomycin or cdtB-expressing plasmid during the 4-weeks experimental period.17

Sonodynamic photodynamic therapy also known as activated cancer therapy, is a recent therapeutic approach

APPLICATION OF SONOPORATION IN DENTISTRY

Gene Delivery
Gene therapy is a procedure in which the introduction of an active gene is achieved into cells to replace a defective gene such that the function of the required cells, which is to be altered, is achieved. “Gene vaccine” against influenza and malaria is developed using a gene therapy.13

Induction of stem cell differentiation of dental pulp into odontoblasts: The main aim of treating dental is to preserve teeth and prolong their function. Nakashima et al.14 in 2003 in Japan reported that the potential to induce reparative dentin formation by pulp capping can be achieved by gene therapy. Sonoporation-mediated gene delivery is done by, a morphogen, growth/differentiation factor 11,
that uses a photosensitive agent with US-activated properties.\(^2\)

**Induction of Apoptosis**
Apoptosis is an organized process of cell death, which occurs naturally for unneeded cells. This process may be used in future for killing cancerous cells and other cells of benign growths before malignant change or size reduction of the growth before surgeries. Ashush *et al.*, 2000\(^{18}\) and Ando *et al.* in 2006\(^{19}\) concluded that exposure of cells to ultrasonic cavitation was shown to induce apoptosis in addition to the conventionally reported instantaneous cell lysis and necrotic disintegration.

**Gene Transduction**
Understanding gene functions is achieved by gene transduction. Sonoporation is an easy and recent advance, which transduces genes into mesenchymal cells without significant damage to target tissues.\(^{20}\) Therefore, sonoporation is effective for gene transduction to study the molecular mechanisms of morphogenesis.

**Recurrent Aphthous Stomatitis**
The use of low intensity us provides a therapeutic benefit by accelerating the wound healing by a change in cellular membrane permeability, stimulation of fibroblasts, increased angiogenesis, and alteration of oral microflora.\(^{21}\)

**Ultrasonic Therapy in Myofascial Pain**
In a study performed by Esposito *et al.*, using US to treat patients with MPDS used pulsed US at a frequency of 1 MHz, a pulse repetition rate of 120 Hz, and intensity of 0.75-2 W/cm for 3-5 min and concluded that US is most successful in alleviating muscle symptoms and less effective in reducing symptoms associated with the disk. Pain relief is achieved by washout of pain mediators by increased blood flow and changes in nerve conduction, which are the result of modified cell membrane permeability that decreases inflammation.\(^{22}\)

**Ultrasonic Therapy for TMD Joint Dysfunction**
A study on 100 patients treated for symptoms of TMD joint dysfunctions and muscle spasm concluded that the ultrasonic therapy is not effective alone in relieving the symptoms of TMD disorders, but effective when used as an additive to other treatment modalities, such as muscle exercises, splint therapy, and heat application.\(^{4}\)

Kropmans *et al.* have done a study by reviewing 24 scientific papers concerning about treatment outcome of various surgical and physical therapies, which included TU for TMD dysfunction and concluded that no difference in success of treatment was noticed.\(^{23}\)

**US Guided Lithotripsy of Salivary Calculi**
Lithotripsy is used for the therapy of calculi. Lithotripsy with a specially designed lithotripter using extracorporeal shock wave for the sialolith of the parotid and submandibular gland was done with successful results using US.\(^{24}\)

**US Therapy in Bone Healing and Osseointegration**
Low intensity pulsed US has been used to accelerate bone healing indicating that US may be used in facilitating the bone regeneration.\(^{25}\) Distraction osteogenesis is considered a successful technique to gain bone and soft-tissue mass in persons with a variety of craniofacial deformities (Figure 3).

A study carried out by El-Bialy *et al.* in New Zealand in which osteodistraction was performed at 3 mm/day for 5 days. After assessing the bone formation by different methods and concluded that bone formation can be achieved by US.\(^{2,26}\)

**Drawbacks**
With so many advantages, the negative effects of this technique are minimal. The process may be time consuming. Minor tingling and burning sensation, irritation of the tissues have been the side effects at site of application.\(^{27}\)

**CONCLUSION**
While in the race of advancing technology, this new avatar of century old US technique is likely to serve as a boom in the field of diagnosis and therapeutic dentistry. With so many applications with minimal need of equipment, this emerging new form of sound energy will revolutionize many forms of treatment. Thus, its simplicity and noninvasiveness will provide a new approach for microinjecting various substances into living tissues. With this we can envision a whole gamut of newer technologies and products in the foreseeable future dentistry.
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