Effects of Low Frequency Ultrasound delivered via the Quostic Wound Therapy System™ on Neuroblastoma (SHSY-5Y) Viability and Morphology

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Purpose

To determine the effects of low frequency ultrasound on cultured human neuroblastoma (SHSY-5Y) viability and morphology.

Introduction

Persistent pain associated with chronic ulcers is a significant clinical problem that afflicts many individuals with open wounds. Pain is now considered the 5th vital sign and also a quality indicator by JACHO in the acute care setting. Wound providers have recently recognized the importance of managing not only procedural wound pain such as occurs at dressing change or during debridement but also chronic pain associated with the wound itself.

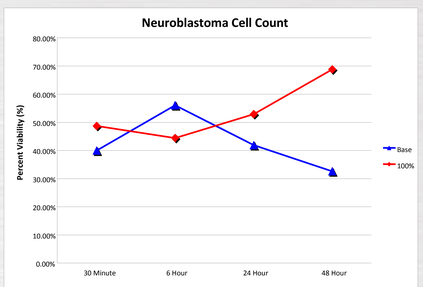
Therapeutic ultrasound delivered at Mega Hertz (MHz) or high frequencies has been used to modulate pain with musculoskeletal injuries for decades. However, recent studies indicate that there may be a role for Kilo Hertz (KHz) or low frequency ultrasound in modulating persistent wound pain.

In the past several years, results from two non-controlled retrospective chart reviews[1,2] indicate that low frequency ultrasound reduced wound-associated pain. These studies involved a variety of chronic ulcerations including lower extremity wounds.

Therefore the purpose of the present study was to determine if low frequency ultrasound delivered by the Quostic Wound Therapy System™ (QWTS) directly affected neuronal viability and morphology

Methods

Human neuroblastoma cells (SHSY-5Y) were obtained from the Wake Forest University cell line repository. The cells were plated and grown in an enriched media to ~90% confluence in a CO_2 Incubator under controlled humidity conditions. Control and low frequency ultrasound (QWTS™) treated cells were exposed to trypan blue in order to determine percent viability. Additional control and treated plates were harvested at 30 minutes, 6, 24 and 48 hours for scanning electron microscopy using standard fixation and staining procedures. Treated SHSY-5Y cells received low frequency ultrasound at 35 KHz at < 1mW/cm^2. Post-treatment, media was aspirated and replaced in both control and treated plates and cells were then incubated according to the timeline above.



Graph 1. Cell viability counts for control, untreated cells (blue line) and low frequency ultrasound (Quostic Wound Therapy System™ (QWTS)) treated cells at 30 min, 6, 24 and 48 hours post-treatment.

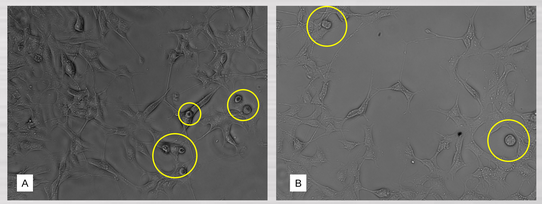


Figure 1. Light microscopy showing control, untreated cells (A) and low frequency ultrasound (QWTS) treated (B) cells 24 hours post-treatment. Note the increased number of nonadherent cells in the control plate versus the QWTS™ treated plate.

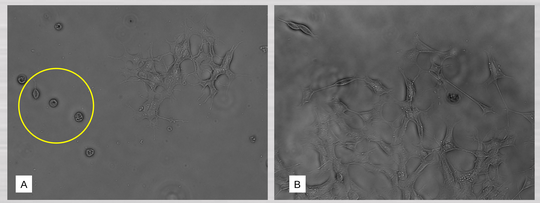


Figure 2. Light microscopy showing control, untreated cells (A) and low frequency ultrasound (QWTS) treated (B) cells 48 hours post-treatment. Note the rounded, nonadhered cells In the control plate versus the increased density of branching cells in the treated plate.

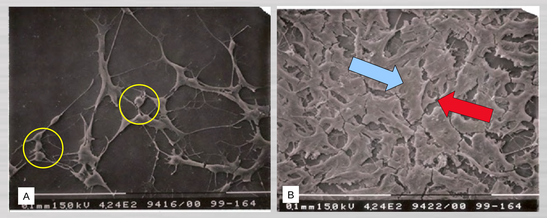


Figure 3. Scanning electron microscopy showing control (A) untreated and low frequency ultrasound (B) treated cells 24 hours post-treatment with the QWTS™. Note all cells in the treatment group have adhered to the plate while several cells in the control plate (see circles) remain rounded and unattached. Cells in the treatment plate have flattened and adhered to the plate with cells exhibiting increased width of cell bodies and projections (blue arrows). Cells in the treatment plate have formed a tissue-like stacking arrangement (red arrows).

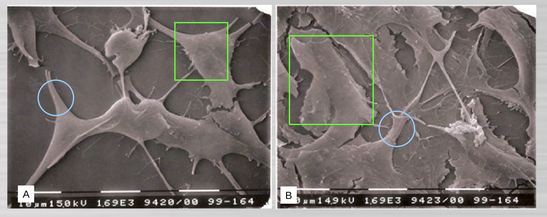


Figure 4. Scanning electron microscopy showing control (A) untreated and low frequency ultrasound (B) treated cells 24 hours post-treatment with the QWTS™. Note the degree of flattening and enlarged cell bodies (green box) and extensions (blue circle) in the QWTS™ treated cells as compared to control, untreated cells.

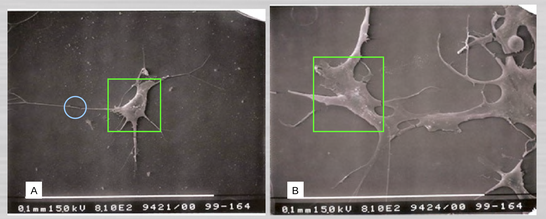


Figure 5. Scanning electron microscopy showing a control (A) untreated and low frequency ultrasound (B) treated cells 24 hours post-treatment with the QWTS™. Note the enlarged cell bodies (green box) and extensions (blue circle) in the QWTS™ treated cells as compared to control, untreated cells. In the QWTS™ treated plated no isolated single cells could be located. Extensive branching and connections were found between all cells.

Results

1. Low frequency ultrasound delivered with the QWTS™ did not decrease cell viability. A trend toward increased viability as compared to controls at 24 hours was detected.
2. Cells treated with the QWTS™ exhibited increased media utilization suggesting an enhanced metabolic response.
3. **Cells treated with the QWTS™ exhibited:**
   - increased cell attachment
   - increased cell body and extension size
   - increased layering of cells in a 3-D structure

Conclusion

Low frequency ultrasound delivered via the QWTS™ appeared to enhance cellular metabolism, growth and the formation of a tissue-like structure with early layering of cells.

While further studies are needed, these results suggest a putative mechanism for low frequency ultrasound modulation of persistent wound pain through a direct effect on neuronal function.

These results also indicate a possible facilitation of neuronal proliferation that may have implications for neuronal regeneration.

Acknowledgement

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References:

1. Gehling ML, Samies JH. The effect of noncontact, low intensity, low-frequency ultrasound on lower-extremity chronic wound pain: a retrospective chart review. Ostomy Wound Management 2007; 53(3):44-40.
2. Bell, A., Cavorsi J. (2008) Noncontact ultrasound therapy for adjunctive treatment of nonhealing wounds: Retrospective analysis. Physical Therapy 88(12):1518-1524.