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Article

Identification of microwave-related changes in tissue using an ultrasound scan

Zain Shariff[1], Nasir Shariff[2]
[1] Curtis Junior High School, University Place, Washington
[2] Cardiology, CHI Franciscan Heart and Vascular Associates, Tacoma, Washington

SUMMARY

Microwave energy (ME) is used in the medical field to denature protein structures, resulting in inactivation or destruction of abnormal cells. Identifying the extent of destruction of abnormal tissue (cancer tissue or tissue with abnormal electrical activity) is essential for accomplishing successful therapy and reducing collateral damage. Our study was an *ex vivo* assessment of the changes on ultrasound scans (US) in chicken tissue exposed to ME. We hypothesized that any changes in tissue structures would be recognized on the reflected ultrasound waves. Ultrasound scans of tissues change with exposure to microwaves with increasing reflection of ultrasound waves. With exposure to microwaves, surface level brightness on the ultrasound scans increases statistically significantly. The findings could be used in heat related (ME and radiofrequency) procedures where clinicians would be able to actively assess lesions in real-time. Further studies are required to assess changes in tissue during active exposure to different types of energies.

INTRODUCTION

Protein structures perform essential functions in the survival of cells, such as regulation of electrolytes, generation of energy, cellular signaling, and structural integrity, and several more (1). With an increase in temperature, proteins unfold and form random configurations resulting in loss of function (2). Microwaves can serve as a source of heat for organic tissue that causes aggregation of protein structures, which can be identified in the form of increased opacification and shrinking of the tissue volume on gross examination (2).

Microwaves are a subset of electromagnetic radiation with wavelengths of one millimeter to one meter, or the equivalent of 300 GHz to 300 MHz (3). Microwave ovens operate using microwaves that force water and fat molecules to rotate, ensuring food is heated and appropriately cooked (4). Medical applications of microwaves are a rapidly developing field. Microwaves result in the destruction of malignant/abnormal cells by rapidly increasing the temperature and causing a denaturation of protein and subsequent loss of self-protective mechanisms in abnormal cells (5). One type of abnormal cell that is denatured using microwaves are abnormal cardiac electrical cells (6). Usually, these electrical cells are in sync causing muscle contractions such as the contraction of cardiac muscles needed to effectively pump blood through the heart (6). However, if there are abnormal cardiac electrical cells present, these can cause the heart to beat irregularly in a condition known as cardiac arrhythmias (6). Identifying the extent of destruction of abnormal tissue is essential for accomplishing successful therapy and to reduce collateral damage.

The amount and duration of microwave energy required for the appropriate amount of destruction of abnormal cells is crucial for a successful therapy. Real-time assessment of degeneration of abnormal tissue would help in achieving this goal. Tissue structures denatured using microwaves result in changes in consistency and color of the tissue, which can be identified visually and by touch sensation. Since the tissue destruction is happening deeper in the organ structures, visual or touch sensation cannot be used in clinical settings. Reflected ultrasound waves have defined information of the tissue structure. Ultrasound is used in clinical practice to define organ structures and identify any abnormalities. Reflected ultrasound waves have a defined signature depending on the tissue they interact with (7). Ultrasounds are a subset of sound waves with a frequency that is 20 KHz higher than humans can perceive, making them inaudible (8). They share physical properties of "normal" (audible) sound, including reflected waves in the form of echoing of the sound (9). The reflected waves contain information about the distance and signal of the object it bounced against. The intensity of reflected ultrasound waves corresponds to the brightness of the image. This property has been used in the medical field to identify organ structures in the body (7). M-Mode is a display of ultrasound waves with relation to time, which provides physicians a monodimensional view of the studied structure (10).

In our study, we sought to define the changes of tissue structures exposed to microwave energy using ultrasound. Can ultrasound be used to identify changes in tissue structures denatured using microwaves? We hypothesized any changes in tissue structures would be recognized on the reflected ultrasound waves. To test our hypothesis, we conducted an *ex vivo* assessment of the changes in ultrasound scans following microwave-based denaturing of chicken meat. We sought to define the changes in external tissue structure following microwave-related destruction of tissue. Ultrasound scans of tissues change with exposure to microwaves with increasing reflection of ultrasound waves. With exposure to microwaves, surface level brightness on the ultrasound scans increases statistically significantly. This knowledge could be used in real time to accurately define the amount of energy and the duration needed to obtain a successful therapy in the management of malignancies and cardiac arrhythmias.

RESULTS

To summarize, the study procedures consisted of first exposing tissue to microwave energy for a variable amount

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of time, examining it with ultrasound M-Mode to allow for high resolution of information at a single axis of study, and quantifying the intensity of reflection.

We studied a total of 10 pieces of chicken tissue cut into 2cm-by-2cm cubes with 300 data points in our project. The highest intensity of reflection was 86.36% at 30 seconds of cooking 5mm deep, and the lowest was 42.61% in uncooked meat, 5 cm deep. The greatest difference in intensity at specified depth was noted between the uncooked meat and 60 sec exposures to microwave (44.69% vs 77.92%) **(Figure 1)**. The intensity of reflection in uncooked meat was on average 44.69±9.51 at surface, 42.61±8.41 at 5 mm, and 48.92±11.74 at 10 mm depth. There was a notable increase of intensity with increase of exposure to microwave energy **(Figure 2)**. There was increased intensity of reflection with a maintained pattern of reflection (compared to uncooked meat) at 15 sec of exposure. With further exposure at 30 and 45 sec, 5 mm depth had the most prominent increase in intensity of reflection. With further exposure at 60 sec, the surface intensity of reflection was most bright **(Figure 1)**. Like the noted changes in ultrasound, it is worth mentioning the visual changes, such as increased opacification, reduction in size, and change in color **(Figure 3)**. The intensity of reflection appeared to decrease in the deep tissue at 60 sec **(Figure 1)**. Using Student T-test to compare the intensity of reflection with uncooked meat as control, there was noted significant increase in values at every level of study at each of the studied cooked timings.

On comparison of varying intensity of reflections at the extreme amounts of time of exposure to microwaves (uncooked vs. 60 sec), the least difference in intensity was at 1 cm (48.92 vs. 57.88, $p = 0.036$) and most at surface (44.69 vs. 77.92, $p < 0.001$). For the data comparing 15 sec vs. 45 sec, the least difference was at 1 cm (62.37 vs. 67.68, $p = 0.095$) and most at 5 mm (59.30 vs. 72.57, $p < 0.001$). When comparing the central duration of exposure (30 sec) with the 15 sec and 45 sec exposures, the least difference was at surface for 30 sec and 45 sec (68.55 vs 67.47, $p = 0.719$) and most was 15 sec to 30 sec at 5mm (59.30 vs. 86.26, $p < 0.001$). When comparing every level of study's amount of reflected ultrasound waves at the surface, 5 mm, and 10 mm, with a defined F-critical value of 2.47 in the two-way ANOVA test, we found that the means were significantly different (surface: F-value = 23.10, 5 mm: F-value = 62.09, 10 mm: F-value= 11.18) and they all had $p < 0.001$.

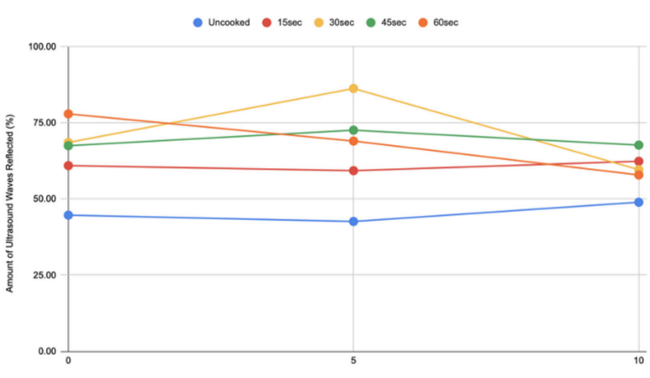

Figure 1: Intensity of reflected ultrasound waves increases at surface levels with longer microwave exposure. Line graph showing intensity of reflection over various depths and lengths of time exposed to microwaves. Pieces of chicken (2 cm x 2 cm) were exposed to microwaves for 0, 15, 30, 45, and 60 sec (n = 2 for each length of time). Intensity of ultrasound reflection was then measured at 0, 5, and 10 mm. Mean Average is recorded in this figure.

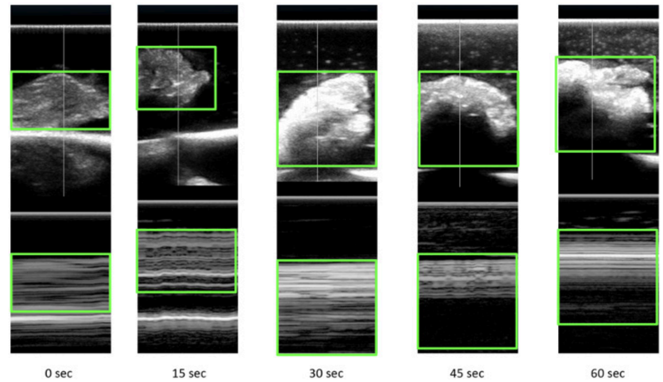

Figure 2: M-mode ultrasound reflection with increasing duration of exposure to microwave energy. The top image is a 2D conventional ultrasound with a line to designate the place on the image that pixels are collected. These pixels are spread out on the bottom image with depth as a vertical axis and time as a horizontal axis. A green box outlines the chicken tissue observed in both the 2D ultrasound and M-Mode.

DISCUSSION

With increasing duration of exposure of organic tissue to microwave energy there is a noted increase in intensity of reflected ultrasound waves. There is noted brighter reflection in deeper tissue initially followed by higher intensity of reflection in superficial tissue with longer duration of exposure. The results of this study supported our initial hypothesis to suggest that the visual changes noted on tissue with exposure to microwave energy can be reproducible with ultrasound findings. A possible explanation would be that most of the ultrasound waves would have been reflected at the surface resulting in very few ultrasound waves in deeper tissue to be reflected. The results of this experiment can be used in heat-related (microwave and radiofrequency) procedures where clinicians would be able to actively assess lesions in real time. Considering that the delivery of the microwave energy is deeper in the tissue and is not accessible to assess visually, present modalities of assessing success use information of strength and duration of energy delivered. With a real-time assessment, we could increase the safety and success of a life-saving procedure.

The practice of medical application of microwaves is increasing rapidly (5). Many applications of microwaves including therapies such as cardiac ablations. A normal heart operates with synchronized electrical signals to perform muscle contractions to pump blood throughout the body (6). However, when the heart electrical signals become abnormal, it can cause irregular heartbeats called cardiac arrhythmias (6). Many of these arrhythmias are dealt using microwave ablations. Using ultrasound scans to monitor an ablation procedure, one can observe the accurate amount of energy needing to be dealt to the site of ablation.

Further studies are required to assess active changes in the tissue during exposure to energies. Additionally, there could be other variables including changes in tissue to active

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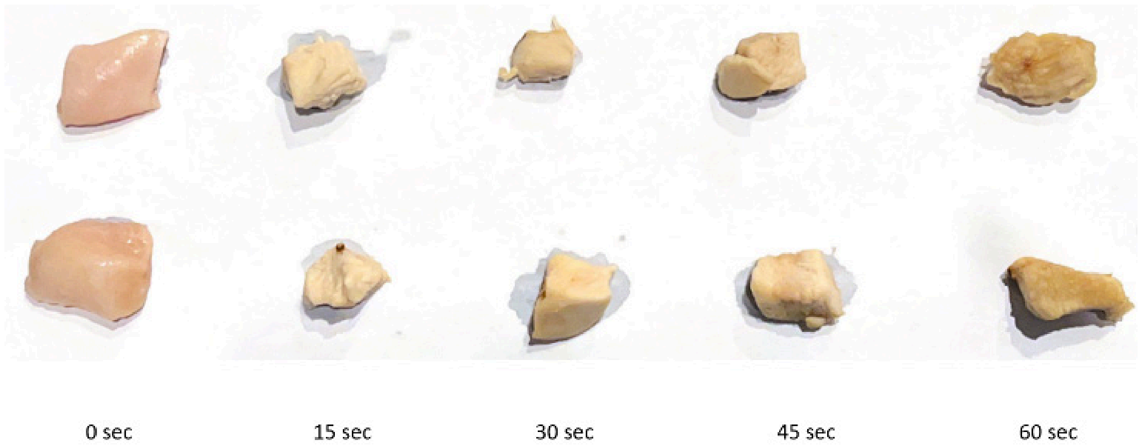

Figure 3: Gross changes in chicken tissue with increasing duration of exposure to microwave energy. Specimens of chicken tissue (n = 10) were exposed to microwaves for a variable duration, two were not exposed at all, listed as "0 sec". Two were exposed to microwaves for 15 seconds, two were exposed for 30 seconds, two were exposed for 45 seconds, and two were exposed for 60 seconds. The tissue tended to change color and lose volume with more time exposed to microwaves.

inflammation happening to *in vivo* tissues and the variability of reflected waves in the situation of surrounding structures. Although, the present modality of ultrasound has the required specifications of the reflected information (as was in our study), a much more precise and denser images with three dimensional ultrasonography would much more likely to be informative and needs to be studied.

MATERIALS AND METHODS

Materials

In this study, chicken meat was obtained from the local store. Chicken tissue was chosen because of its similar properties to human muscular tissue and its widespread usage in the medical field for *ex vivo* testing. A sharp knife was used to prepare and cut the pieces and a conventional microwave oven to expose the tissue to microwaves. A 3.5 MHz 2D M-Mode Ultrasound was purchased for this study. These ultrasound machines are reliable and allowed us to gather data from one point of chicken tissue and keep track of depth of the ultrasound images. To complement the ultrasound machine and collect reliable images, a small container was filled with water and a 5GB USB to store and transfer images from ultrasound machine to computer. For post-processing and data collection (Google Sheets), we used the Paint App on Windows 10.

Methods

All safety procedures were executed to prevent the introduction of potential bacteria in the chicken tissue, including wearing gloves, a mask, and goggles. Hand washing was mandatory and frequent, and all waste was disposed of with other food waste.

Ten similar slices of chicken tissue were cut with over 2 cm in thickness in all dimensions as measured in raw status. This was to make sure that with tissue shrinkage with exposure to microwaves, the resulting tissue was larger than ten millimeters in all dimensions for data gathering. Of the 10 slices of tissue, 2 slices were kept to the side and noted as "uncooked meat". Another 2 were placed in the microwave for 15 sec, another 2 for 30 seconds, another 2 for 45 sec, and the last 2 for 60 sec.

After the tissues were exposed, the ultrasound was switched on with M-Mode, depth 2.5 cm, gain at 92, and tints off. One by one, each specimen was placed in a small container filled with water and ultrasound images were taken. A desirable ultrasound image would have the chicken tissue in sight on the 2D ultrasound and the M-Mode image "filled" **(Figure 2)**. We saved the images on a USB and opened them on our Windows 10 computer via Paint App.

On the Paint App, we viewed the image and located the surface depth, five millimeters deep, and ten millimeters deep. A space of one millimeter by one millimeter was used to define the region of interest. Our ultrasound images provided such measurements on the side of the M-Mode image. The highest intensity in the defined region was used for data location and a total of 10 locations at a defined depth with an equal distance in between were collected. A color picker tool was used to obtain the intensity of the location. We used the RGB (Red, Green, Blue) value of our point to calculate the intensity of the reflected waves. The three values of the RGB tuple should be equal; if they were not equal it was a high chance tints were on. The following equation was used:

$$y = \frac{20}{51}x$$

The number that was common between the three RGB values was used in the following equation as x and y was an output in a percentage. These percentages were collected in a spreadsheet for data interpretation. Two-Way ANOVA, Student T-Test, and Standard Deviation is used for data interpretation.

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