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ORIGINAL PAPER

Hyperthermia Therapy Monitoring with Guidance from B-Mode Ultrasound

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ABSTRACT

Local hyperthermia treatment for cáncer therapy has high potential to be used in conjunction with the other existence treatment as such chemotherapy, clinical surgery and radiation. B-Mode ultrasound has shown great potential for local hyperthermia monitoring method, as it is nonionizing, convenient, and has relatively simple signal processing and it offers a good spatial resolution. The aim of this study is to compare the most optimum B-Mode ultrasound parameters in normal and pathological tissue. The subjects were dissected and exposed to hyperthermia at 37◦C, 40◦C, 45◦C, 50◦C and 55◦C. Result shows that for mean grey scale in normal and pathological tissue, 37◦C and 40◦C were optimum temperatures with corresponding values of 188.08 and 199.26. Meanwhile for pixel deviation analysis, at 45◦C and 55◦C were the best temperatures are observed in both tissues with pixel deviation of 57.27 and 57.25. Additionally, it is proven with an accuracy of testing to validate the data using ANN, about 88.89% and 81.25% was achieved.

INTRODUCTION

Randomized trials on the benefits of hyperthermia therapy have been demonstrated over the past 20 years [1]. Notably, hyperthermia is a cancer therapy that elevated the tumor to cytotoxic temperatures from 41◦C to 45◦C in order to aid in their control [2]. Also, hyperthermia has shown to be a viable adjunct to replace the current clinical treatment including radiotherapy and chemotherapy [3]. It has been applied in several diseases including breast cancer [4], glioblastoma [5], head and neck cancer [6], hepatocellular carcinoma [7], lung cancer [8]. However, other type therapy such as high-temperature thermal ablations also been searched and investigated [9]. However, in 2012 there were 5,410 new breast cancer in Malaysia reported by The International Agency for Research in Cancer (GLOBOCAN), in which 50% of Malaysian women who were diagnosed with breast cancer at their early age whilst 20% of women in developed countries were diagnosed before the age of 50 [10]. Some tumors, however, are very aggressive and grow much faster and require immediate treatment. Tabar et al. have demonstrated that survival rate is greatly improved if breast abnormalities such as breast masses are detected at the earliest stage which is concurred by Andersson and Ryden [11,12]. Hence early treatment of breast cancer is very important at this time to get rid of the cancer and perhaps it can save the life.

The lack of precise information available in guiding therapy, it has been reported a major limitation in thermal therapies [13]. In order to meet the capability and goals of current and forthcoming heating device and system, the temperature distribution is routinely measured invasively. To meet satisfactory temperature distributions in order to assess thermal dosimetry properly, the limited number of measurement distribution could be avoided [14]. With multiple heating devices, it has boosted the demand for temperature measurements that could provide detailed feedback about temperature distributions. Thus, it improves the ability to deliver the heat source consistently by achieving effective temperature value [15].

The application of hyperthermia therapy is now growing rapidly in the medical field especially for treatment of benign and malignant solid tumors [16] as an innovative and less invasive therapy method. Hyperthermia therapy is one of the cancer treatment among surgery, chemotherapy, radiation therapy and immunotherapy [17]. It involves the changes and increases in body temperature over the threshold temperature of an organism [17]. In other words, hyperthermia is a process of delivering heat to tumor tissue until exceeding its durable temperature by some kinds of heating methods, including traditional water heating and microwave coagulation. However, during the procedure, the temperature of tissues must be kept within a suitable range [18] for ensuring the therapy can be carried out effectively and safely. Hyperthermia treatment makes use of artificial heat, usually in the range of 40oC to 60oC, from external sources to destroy cancerous cells or to prevent their further growth [19]. Also, as reported by the previous study the temperature range of hyperthermia therapy is from 40 to 48◦C and is maintained at the treated site for a period of one hour or more [20]. In some cases, hyperthermia therapy is used as adjuvant therapy [20] with another therapy method such as radiation to fasten tumor regression rate than radiation alone.

Hyperthermia monitoring with the guided of Magnetic Resonance Imaging (MRI) is currently the gold standard in the clinical setting and it is based on the concept of shift of proton resonance frequency. Its function is primarily for surgeons to perform a real-time progression assessment of in-vivo tissue necrosis [21]. Although MRI has shown to be capable in visualizing very clear and anatomically correct images, it is found that its performance degrades during hyperthermia treatment due to small temperature interval changes [22]. In contrary to the ultrasound system, A-Mode and B-Mode ultrasound are good approaches in monitoring thermal therapy. It is sensitive to temperature change, required simple signal processing and

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provide good spatial resolution [23]. It is common imaging methods used to evaluate tumors in the thyroid, breast, prostate, liver, pancreatic, ovarian, uterine and kidney, and is frequently used to guide biopsies [24]. An advantage B-Mode ultrasound to monitor temperature during hyperthermia monitoring is that this technique is relatively inexpensive, portable and can be easily employed in almost any current heating monitoring system with little concern about system compatibility. Ultrasound benefits from its portability, accessibility, low cost, non-ionizing, and compatibility with other medical equipment [21]. Technically, the monitoring approaches are based on variation in acoustic speed, [25], the energy of backscattered pulsed ultrasound and acoustic nonlinear parameter imaging [26].

Therefore, this study was conducted to investigate the effectiveness of B-Mode ultrasound in monitoring hyperthermia with a variation of temperature setting. The aim of this study is to develop a special transducer to be embedded to the hyperthermia system. This study will help to improve the overall hyperthermia therapy reliability to be accepted in clinical practice while assisting physicians and medical practitioners in monitoring hyperthermia treatment efficiently with minimal intervention.

MATERIALS AND METHOD

Animal Handling

All experiment protocols dealing with animals were conducted in accordance with the rules and regulations which have been approved by the institutional review board of Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC), Selangor Malaysia, with granted ethical endorsement {UTM/2013/MAHEZA/17 JULY/527}. In this study, female Sprague Dawley Rat was selected because they are easier to handle and manipulate. During adaptation period, the rats were left in the laboratory environment for one week. There are 10 normal and 30 DMBA-induced virgin female rats were kept for this study. All rats were placed in each polypropylene cage complete with enough wood shavings as their bedding, foods ad libitum and drinks. All the polypropylene cages were placed in a room at ambient temperature and controlled by automated 12 hour light/dark cycle. The weight of each rat was measured weekly, which it is in the range of 150 g to 330 g..

Experimental Procedure

Figure 1 shows the block diagram of heating exposure of this experiment. B-mode ultrasonic scanner was used to capture the scanning images during the experiment. The sample tissue was heated at different temperatures located between the ultrasound transmitter and receiver at 37℃, 40℃, 45℃, 50℃ and 55℃ respectively. The images from B-mode ultrasound was further processed in Matlab, offline environment for the purpose of image processing via active contour method to segment the target area of the normal and pathological tissue condition.

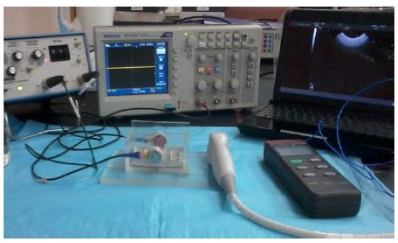

Fig 1. Experimental set up during hyperthermia therapy.

Cancer Induction

The 30 virgin female rats were treated a with a single dose of DMBA 1mg, dissolved in 0.2ml sesame oil purchased from Sigma-Aldrich by gastric gavage/weekly for 15 weeks to initiate breast cancer. DMBA is a chemical reagent known as polyaromatic hydrocarbon for initiating the breast cancer [27,28]. As reported in a clinical study, the outbred Sprague Dawley (SD) rats are among the most sensitive to carcinogen-induced mammary cancers [29]. It is powerful organ- specific laboratory carcinogen purposes and immunosuppressor [30]. The use of DMBA in an animal model has shown to have a closer mimic to the human breast as it plays a role in metabolites with capacity towards the damaging of DNA which is the prime factor in the initiation of carcinogenesis as reported by the previous study [6]. The formation of mammary cancer via DMBA injection can be seen clearly without another contributing factor such as hormonal restriction as demonstrated by the previous researcher [31]. Hence, DMBA has been chosen as one of the chemical reagents in inducing and initiating mammary cancer for the animal model either mice or rat as it provides the effective reaction upon the injection. Mammary glands were palpated weekly for mammary tumor detection. Tumor onset, number (multiplicity) and growth were recorded when possible. Figure 2 shows the excised tumor from the subject.

Fig. 2 Extraction of tumor from each rat.

Tissue Preparation

The anesthetic drug was given to the rat intravenously via the mouse tail. Before dissection, the fur around the breast rat area was shaved. Surgery procedure was continued by harvesting normal and pathological mammary tissue to an approximately 6cm x 6 cm square shapes, with a thickness of 2.5 mm from each normal and DMBA-induced virgin female rat during their sleeping time. Next, the rats were killed by using drug overdose method which had been approved by the animal ethics committee. The mammary tissues of normal and DMBA-induced virgin female rats were heated using water bath in the ex-vivo settings at different temperatures which are 37℃, 40℃, 45℃, 50℃ and 55℃ for 5-10 minutes to mimic a real hyperthermia procedure. After the dissection, the tissue samples were immediately placed inside the ice box for tissue hygiene, avoiding tissues death and maintain the structure before exposing to hyperthermia. After 5-10 minutes, the tissues were continuously exposed to 6 MHz frequency of B-Mode.

B-mode eature Extraction

All the images collected from B-Mode ultrasound were saved in the form of Joint Photography Experts Group (JPEG). The data from this modality was further analysed in Matlab offline environment. The image processing was done in which the images were first loaded in Matlab for pre-processing and enhancement purposes. Next, the images were cropped for the purpose of removing unwanted region to be segmented. The cropping images later were considered as the finalised image for plotting the coordinate on the image itself. The coordinate were plotted clockwise on the images accordingly. Later, the segmentation processing via snake starts to process for indicating the boundary of the targeted region. Finally, the boundary of the targeted area was identified and segmented. The outcomes of every segmented image were saved for further statistical analysis calculation and performance evaluation such as the mean grey scale of pixel intensity and pixel deviation of the segmented boundary. Figure 3 shows the outcome of images segmentation workflow in this study. Firstly, the images were loaded in Matlab for pre-processing including image enhancement, contrast adjustment and cropping as shown in figure (a). In figure (b), it shows the image with initial contour, by means the image after plotting the

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coordinates surrounded the boundary. The image of external energy was shown in figure (c) by means of translating from the initial contour. Meanwhile in figure (d) is the outcome after snake movement identification of boundary. Finally, the segmented region was shown as in figure (e) showing the area of interest successfully segmented.

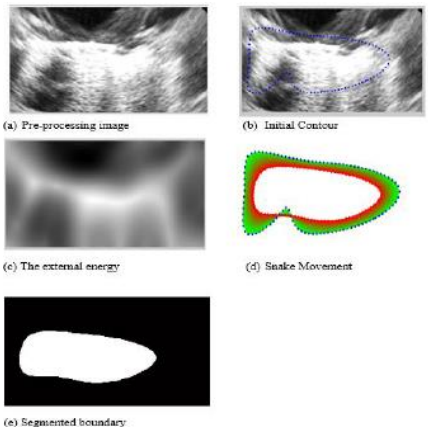

Fig 3. Active contour segmentation workflow

An active contour or snake is a parametric curve expressed as $Z(s) = X(s), Y(s)$, where$0 \leq S \leq 1$. The parameter x and y are the coordinate of the vertices, v and are functions of the normalized arc length s. The Snake has a dynamic behavior that deforms from an initial position and converges to the boundary of the object in the image [32]. It moves through the domain of the image by minimizing its energy function. The *E*snake is defined as;

$$Esnake = \int_0^1 [Eint(Z(s)) + Eext(Z(s))]ds \quad (1)$$

The internal energy function is;

$$Eint(Z(s)) = \frac{1}{2}\{\alpha|Z'(s)|^2 + \beta|Z''(s)|^2\} \quad (2)$$

The internal energy function Eint(Z(s)) is computed based on the local shape of the curve v(s) and is responsible for determining the continuity and the smoothness of the curve. The parameter α and β are the coefficients of the internal energy function. The parameter α is the elasticity parameter. For a large value of the curve becomes very straight between two points. The parameter β is the rigidity parameter and for a large value of β, the curve becomes smooth. On the other hand, the external energy function Eext (Z(s)) is derived based on the image information and it drives the curve to the boundary of the object [33]. Different types of Snakes use a different type of external energy function. By calculus of variation, Equation (1) is minimized by solving the associated Euler's Equation as follows;

$$-\alpha Z''(s) + \beta Z^{(4)}(s) + \nabla Eext(Z(s)) = 0 \quad (3)$$

In B-Mode images, for the purpose of feature extraction, first-order statistical parameters on mean grayscale (MSG) and pixel deviation of the grayscale are measured using grey level histogram method [24]. Firstly the calculation of the occurrence of probability grey scale could be done and it can be denoted as;

$$P(rk) = \frac{nk}{n} \quad (4)$$

where *n* is the total number of the pixels in an image. Therefore,

$$\sum_{k=0}^{L-1} P(rk) = 1 \quad (5)$$

The MGS is the mean of the gray scale for pixels in an image. This can be calculated as;

$$MGS = \sum_{k=0}^{L-1} rk.P(rk) \quad (6)$$

The STD measures the dispersivity of the gray scale from the MGS. It can be calculated as;

$$STD = \sum_{k=0}^{L-1}(rk - MGS).P(rk) \quad (7)$$

Development of Artificial Neural Network (ANN)

An artificial neural network B-Mode for breast cancer classification was developed in this study. There are four stages in this ANN development. In the first stage, data massaging and data sampling were processed in a spreadsheet format in Microsoft excel. A total of 130 of B-Mode data were obtained during the experiment and were used as input and output of ANN. The input data were divided into three different group namely training (N=78), testing (N=36) and validation (N=16). The range of target output is from 0 to 1, the value of '1' indicating normal tissue condition while '0' indicates abnormal tissue condition. The mean grey scale, pixel deviation, variance and area are the input parameters that were fed into ANN development. The data were prepared separately in a spreadsheet in Microsoft Excel and then were exported into the Matlab workspace for further processing for training, testing and validation purposes.

RESULTS AND DISCUSSION

Mean Grey Scale and Pixel Deviation of Pixel Intensity

The results of this study were summarized as in Figure 4 and Figure 5 respectively. Figure 4 and 5 shows the average mean of pixel intensity and pixel deviation of B-Mode images in normal and pathological tissue. The tissue was heated at various temperatures before and after hyperthermia. In Figure 4, for normal tissue condition, the highest mean grey scale of pixel intensity was observed at temperature value of 37◦C with a pixel intensity value of 188.08 while the lowest mean grey scale of pixel intensity was 183.07 at 55◦C. The mean grey scale of pixel intensity in pathological tissue of 192.15, 199.26, 195.10, 195.82 and 193.18 were observed at the temperature of 37◦C, 40◦C, 45◦C, 50◦C and 55◦C individually. In summary, in normal tissue condition, a temperature value of 37◦C was chosen as an optimum temperature dependent seems the mean grey scale of pixel intensity is more pronounce than the other values, meanwhile a temperature value of 40◦C was chosen as an optimum temperature dependent in pathological tissue as it produced the highest pixel intensity for B Mode analysis with respect to the mean of pixel intensity in monitoring hyperthermia therapy for both tissue condition.

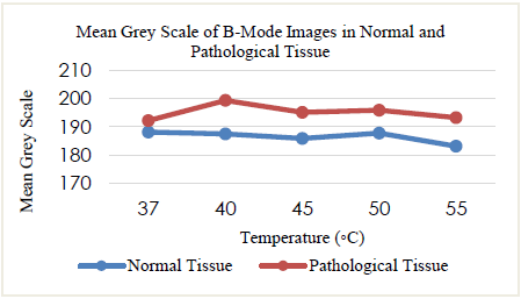

Fig 4. Mean grey scale vs temperature in normal and pathological tissue

Based on Figure 5, the average pixel deviation at body temperature in normal tissue which is before hyperthermia was 55.74. Meanwhile, the average pixel deviation in normal tissue at 40◦C, 45◦C, 50◦C and 55◦C after hyperthermia was analyzed with corresponding values of 56.84, 57.27, 53.49 and 54.79 individually. From statistical data analysis also, the average pixel deviation of B-Mode images in pathological tissue at 37◦C was found to be 53.03. After hyperthermia, the highest pixel deviation was analyzed at temperature value of 55◦C with a corresponding value of 57.25 while at temperature values of 40◦C, the average pixel deviation tends to be 48.89 which indicates the least pixel deviation as compared to the others pixel deviation values. Hence, in comparison to both condition, the temperature value of 45◦C and 55◦C were chosen as optimum temperatures dependent on normal and pathological tissue condition

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for hyperthermia therapy monitoring with respect to the average of pixel deviation of both tissue condition. Several studies have reported that the texture features of B-mode ultrasonic images have certain relationships with tissue temperature [34]. Pousek et al found that both the mean of the gray scale (MGS) and gradient scale value increased with tissue temperature. They also showed that the feasibility of temperature monitoring is high for B-mode ultrasonic image processing in hyperthermia [35]. Wu et al1 proved the significant difference in the gray value of treated regions in a B-mode image before and after heating [36].

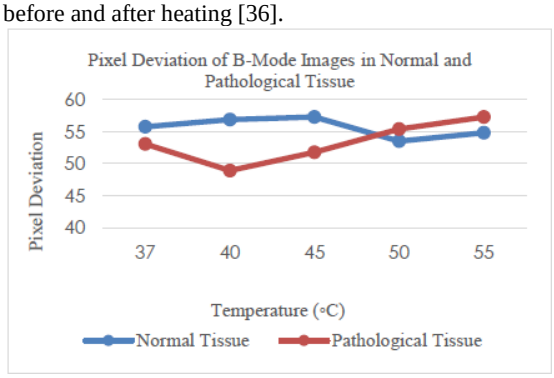

Fig 5. Pixel deviation vs temperature for normal and pathological tissue

Correlation Plot between Mean Grey Scale and Pixel Deviation

Figure 6 illustrates the correlation between mean grey scale and pixel deviation of B-Mode ultrasound obtained from the experiments. Mean grey scale level changes against pixel deviation is more sensitive in pathological tissue samples with a positive correlation coefficient of 0.6428, while it records a value of 0.0671 when associated with pixel deviation changes in normal tissue samples. This particular finding specifies that as the hyperthermia treatment is conducted, the image occurring in the pathological tissue can be monitored using mean grey scale level, prior to further biological changes taking place within the same tissue.

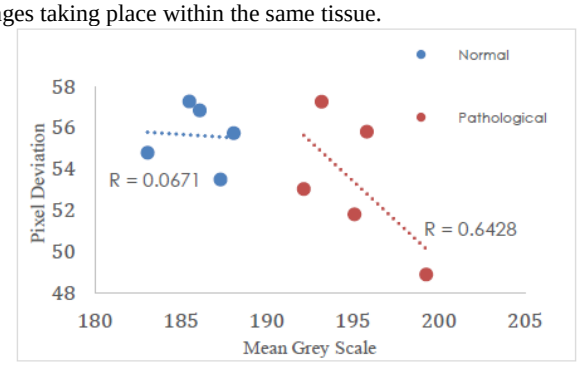

Fig 6. Sensitivity plot (pixel deviation against mean grey scale)

CONCLUSION

In summary, in this study, the feasibility of B-Mode ultrasound for real time and accurate thermal therapy monitoring method was investigated. The temperatures value of 37◦C and 40◦C were more pronounced in normal and pathological tissue condition for calculation mean of pixel intensity, meanwhile, for average pixel deviation, the temperature value of 45◦C and 55◦C were chosen as optimum temperatures dependent of normal and pathological tissue condition. This study was revealed in which the calculation of all parameters of B-Mode including mean grey scale of pixel intensity and average pixel deviation via active contour algorithm was able to segment and it is sensitive to tissue structure with the variation of temperatures during hyperthermia monitoring. It was proven with the percentage accuracy of testing to validation data using the artificial neural network, about 88.89% and 81.25% was achieved and the success of this study will help to provide a simple and safe method in monitoring local hyperthermia therapy using B-Mode ultrasound.

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