EFFECT OF CONTINUOUS THERAPEUTIC ULTRASOUND ON BREAST CANCER CELLS

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To my beloved parents and my dear brother Mohammad

No one has ever been given more loving and unconditional support than you.

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ABSTRACT

The aim of this study is to investigate the potential of continuous therapeutic ultrasound as an alternative therapy to treat breast cancer disease. The study starts with the optimization of the cell seeding numbers, followed by exposure the cells with the ultrasound with two different doses, then MTT assay was used to investigate the cells viability/toxicity. The doses of therapeutic ultrasound were applied on breast cancer cell lines (MCF-7) and normal cell lines (Chinese Hamster Ovary). In this treatment, cells were exposed to the therapeutic ultrasound for three days interval. Based on the MTT assay, it was revealed that exposure of therapeutic ultrasound shows an inhibition effect to MCF-7 cells and less harm on CHO cells. However, when comparing both doses, it shows that dose 2 (3MHz, 0.2W/cm^2, 10 minutes, continues cycle, distance from the bottom to the plate is 0.5 cm) have a better effect than dose 1 (3MHz, 0.1W/cm^2, 10 minutes, continues cycle, distance from the bottom to the plate is 0.5 cm). These results indicated that continuous therapeutic ultrasound has an effect in inhibiting the breast cancer cells growth and has less harm effect on normal cell.

ABSTRAK

Tujuan kajian ini ialah untuk mengkaji potensi terapeutik ultrasound berterusan sebagai terapi alternatif untuk mengubati penyakit kanser payudara. Kajian ini dimulakan dengan pengoptimuman nombor pengkulturan sel diikuti oleh pendedahan sel melalui ultrasound dengan dua dos yang berbeza. Selepas itu, MTT assay telah digunakan untuk mengkaji kesan toksik ke atas sel. Dos-dos ultrasound terapeutik telah digunakan pada sel kanser payudara (MCF-7) dan sel normal, (Chinese Hamster Ovary (CHO)). Dalam kajian ini, sel-sel telah didedahkan kepada ultrasound terapeutik selama tiga hari. Berdasarkan kepada MTT assay ia telah menunjukkan bahawa pendedahan ultrasound terapeutik menunjukkan kesan perencatan kepada sel-sel MCF-7 dan kurang perencatan kepada se-sel CHO. Tetapi, apabila dibandingkan kedua-dua dos ini, ia menunjukkan bahawa dos kedu (3 MHz, 0.2W/cm^2, 10 minit, kitaran yang berterusan, jarak dari bahagian bawah plat adalah 0.5 cm) mempunyai kesan yang lebih baik berbanding dengan dos 1 (3MHz, 0.1W/cm^2, 10 minit, kitaran yang berterusan jarak dari bahagian bawah plat adalah 0.5 cm). Keputusan menunjukkan bahawa ultrasound terapeutik berterusan mempunyai kesan dalam merencatkan pertumbuhan sel-sel kanser payudara dan kurang perencatan pada sel-sel normal.

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LIST OF ABBREVIATIONS

CBE	-	clinical breast examination
BSE	-	breast self-examination
MHz	-	Mega Hertz
W/cm^2	-	Watt per centimeter square
MCF-7	-	Breast cancer cell line
CHO	-	Chinese Hamster Ovary cell line
MTT	-	3 - [4,5 - dimethylthiazol-2-yl] -2,5-tetrazolium bromide diphenyl
nm	-	nanometer
RPMI 1640	-	Roswell Park Memorial Institute Medium
FBS	-	Fetal Bovine Serum
PBS	-	Phosphate Buffer Saline
Pen Strep	-	Penicillin-Streptomycin
ATCC	-	American Type Culture Collection
ml	-	milliliter
°C	-	degree Celsius (centigrade)
mg	-	milligram
µm	-	micrometer
M	-	Molar
HCl	-	Hydrochloric acid
mM	-	milli molar
CO_2	-	Carbon dioxide
cm	-	centimeter
µl	-	micro liter

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Every year, people are dying from various diseases such as; cardiovascular disease, cancer, diabetes and chronic lung diseases and more than 10 million people are diagnosed with cancer every year with the rate might increase up to 50% by 2020 (Luxford *et al*., 2009). Cancer is an abnormal cell (malignant cells) in the body which happen due to the uncontrolled growth (Giuliano *et al*., 2011). It was reported that every year over 1.15 million women in the world are being diagnosed with breast cancer and 502,000 die from the disease (Montazeri *et al*., 2008). In Iran, the incidence of the breast cancer disease in young age is rising, with advanced stage of the disease (about 10 years) compare with their western counterparts (Montazeri *et al*., 2008).

Breast cancer is one of the most common disease in females (Montazeri *et al*., 2008; Wu *et al*., 2003) with a life-time risk, 1 out of 8 women diagnosed with breast cancer (Montazeri *et al*., 2008). There are two major types of breast cancer; ductal carcinoma and lobular cancer. Ductal carcinoma commence in the tubes which milk move from the breast to the nipple, in addition, most breast cancers are occurred based on this type. Lobular cancer, commence in lobules that it is part of breast which produce

milk (Giuliano *et al*., 2011; Warner, 2011). However, breast cancer proceed in two extensive class; non-invasive and invasive, and breast cancer can commence hardly in other location of the breast. Invasive conduct with cancer spreading from milk duct or lobule to other tissue in the breast. In contrary, non-invasive means it has not yet invaded other breast tissue (Parkin *et al*., 2005).

Early detection of breast cancer is critical for reducing any fatal consequences. While screening according to breast self-examine associated with various factors in society (age, education, information about breast cancer) and this could control breast cancer incidence (Parkin *et al*., 2005; Montazeri *et al*., 2008). Symptoms of cancer growth may be comprised with changing the shape or size of the breast or nipple, nipple discharge, in addition, symptoms in advance breast cancer may be reveal; breast pain, bone pain, swelling of in the armpit (Montazeri *et al*., 2008; Hanan *et al* ., 2009).

Screening Mammography, clinical breast examination (CBE) and breast self-examination (BSE) has been suggested as an effective method for early detection (Montazeri *et al*., 2008). In addition, screening based on mammogram is an expensive method and it is ineffective for female younger than of 50 years (Montazeri *et al*., 2008). A clinical breast examination (CBE) is a simple and inexpensive method to breast detection with variable interpersonal interpretation (Jatoi, 2003). For this exam, special consideration will be given to abnormalities in shape or size of the breast, changes in the skin of the breasts including rashes and redness of nipples for any discharge and area under both arms. Then, the examiners concentrate on the location of any lumps of softness, and whether such lumps are attached to the skin or to the deeper tissues. Nevertheless, it has been shown that the clinical breast exam could detect around 60% of breast cancer detected by mammogram and some breast cancers cannot detect by mammography (Montazeri *et al*., 2008; Weiss, 2003). Breast self-examination (BSE) is a regular examined by women when they looked and felt both breasts by herself for possible lumps (Lam *et al*., 2008). It is an important way of finding breast cancer at the

early stage, when it is more likely to be treated successfully. BSE has conducted with the potential of breast cancer detection with less facilities rather than mammography (Montazeri *et al*., 2008).

One of the advance breast cancer treatments is surgery; this method involves removes cancerous tissue which includes lumpectomy and mastectomy. Lumpectomy removes the cancerous tumor; anyhow, include normal breast tissue removed around a cancerous tissue. In contrary, mastectomy removes all or part of the breast. Further advice involves the removal lymph from underarm (axillary) for achieve more information on stage of disease (Parkin *et al*., 2005). For radiation therapy technique based on using the X-rays, gamma rays and other radiation sources to kill the cancerous cell tissues and shrink the tumors. Radiation therapy may include external beam radiation (it comes from a machine outside the body), also called external radiation therapy and internal radiation (it comes from radioactive material placed in the body near the cancer cells). The side effects of radiation therapy are occurred because high doses of radiation used to kill the cancerous cells can also damage healthy cells in the treatment area (McGahan *et al*., 2000). The side effects may be cause extra affect, meanwhile, patients also receive chemotherapy, during or after radiotherapy. It shows different effect on each person, such as fatigue, vomiting and skin changes that may include dryness, and, itching. Many of these side effect features disappear within two months after radiation therapy is completed. Furthermore, late side effects may first occur six or more months after radiation therapy completely finished (McGahan *et al*., 2000). Patients who exposed to radiation may comprise with late side effect, namely, infertility, joint problems and mouth problems. Other cancer treatment is chemotherapy which related to medication with drugs to treat the cancer. As a result for the side effect of chemotherapy, patients lose their hair, loss of appetite, nausea, vomiting, diarrhea, or mouth sores. In additional, hormone therapy is a cancer treatment which removes hormones or blocks their action and stops cancer cells from growing and the side effect of this treatment is like the other (Chlebowski *et al*., 2010).

Recently, a new approach for treating cancer was discovered as another way to minimize overall disadvantage of using treatment of surgery, chemotherapy, radiotherapy and hormone therapy (Yu *et al*., 2004; Böhm *et al*., 2000). Prospective of ultrasound for cancer treatment was published in 1933 which conducted with Ehrlich's carcinoma (Haar, 1999; Haar 2007).

Therapeutic ultrasound is refers to the mechanical wave that usually used in a treatment that specifically for tissue repair (Speed, 2001). It is also, has a different approach when compared to the ultrasound imaging that widely use for the pregnancies (Haar, 2007). Therapeutic ultrasound frequency has a range 0.75-3 MHz, however most ultrasound machines set at a frequency of 1 or 3 MHz (Speed 2001). In this study, the approach is to inhibit the growth of cancer cells rather than for the tissue repair. To achieve this goal, low frequency of therapeutic ultrasound is used to treat on the breast cancer cells. This is because, low frequency of ultrasound waves have greater depth of penetration but are less focused. Furthermore, high penetration of ultrasound waves is seen in tissue rich in fat (such as breast tissue). This feature can make the therapeutic ultrasound is an alternative treatment for breast cancer (Speed 2001; Al-Bataineh *et al*., 2011).

1.2 Problem Statement

Cancer is a disease where the cells have an abnormal growth. For its treatment, the growth of cells should be inhibiting, remove the malignant tumor, suppress the gene that code for the cancer and others. Ultrasound treatment may be able to inhibit cancer cell growth as an alternative treatment. Thus, a study carried out on the sufficient

intensities and frequencies of ultrasound which has more effect on inhibitor breast cancer cell growth without or less affecting on other cells.

1.3 Objective of study

The overall objectives of the study are:

1) To optimize cell seeding density for normal cell line (CHO) and breast cancer cell line (MCF-7).

2) To investigate the effect of two doses of therapeutic ultrasound on MCF-7 (cancer cell) and CHO (normal).

3) To analyze which dose has a better effect on MCF-7 with no/less harm on CHO cells.

1.4 Scope of the study

This study was conducted to identify which dose has a better effect to inhibit breast cancer cells growth and at the same time has less harm to the normal cells. Cells that have been treated with the therapeutic ultrasound will then be analyzed by using the MTT assay to check if there is any toxicity or inhibition effect.

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1.5 Significance of the study

Since breast cancer is one of the leading health threats for human throughout the world, the therapeutic ultrasound treatment is expected to be an alternative remedy to help in fighting the breast cancer diseases.

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