

In vitro Study of Laser Diode Radiation Effect on the Photo-Damage of MCF-7 and MCF-10A Cell Clusters

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Abstract—Breast Cancer is one of the most considerable diseases in the United States and other countries and is the second leading cause of death in women. Common breast cancer treatments would lead to adverse side effects such as loss of hair, nausea, and weakness. These complications arise because these cancer treatments damage some healthy cells while eliminating the cancer cells. In an effort to address these complications, laser radiation was utilized and tested as a targeted cancer treatment for breast cancer. In this regard, tissue engineering approaches are being employed by using an electrospun scaffold in order to facilitate the growth of breast cancer cells. Polycaprolacton (PCL) was used as a material for scaffold fabricating because of its biocompatibility, biodegradability, and supporting cell growth. The specific breast cancer cells have the ability to create a three-dimensional cell cluster due to the spontaneous accumulation of cells in the porosity of the scaffold under some specific conditions. Therefore, we are looking for a higher density of porosity and larger pore size. Fibers showed uniform diameter distribution and final scaffold had optimum characteristics with approximately 40% porosity. The images were taken by SEM and the density and the size of the porosity were determined with the Image. After scaffold preparation, it has cross-linked by glutaraldehyde. Then, it has been washed with glycine and phosphate buffer saline (PBS), in order to neutralize the residual glutaraldehyde. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for (MTT) results have represented approximately 91.13% viability of the scaffolds for cancer cells. In order to create a cluster, Michigan Cancer Foundation-7 (MCF-7, breast cancer cell line) and Michigan Cancer Foundation-10A (MCF-10A, human mammary epithelial cell line) cells were cultured on the scaffold in 24 well plate for five days. Then, we have exposed the cluster to the laser diode 808 nm radiation to investigate the effect of laser on the tumor with different power and time. Under the same conditions, cancer cells lost their viability more than the healthy ones. In conclusion, laser therapy is a viable method to destroy the target cells and has a minimum effect on the healthy tissues and cells and it can improve the other method of cancer treatments limitations.

Keywords—Breast cancer, electrospun scaffold, polycaprolacton, laser diode, cancer treatment.

I. INTRODUCTION

BREAST cancer is considered to be the most common cancer among women in the United State and is the second leading cause of death in women [1]. Surgery, chemotherapy, radiation therapy, and molecular agents are the

current therapeutic methods for breast cancer treatment. Although these common treatment methods improve the survival rate, they also incorporate side effects such as targeting healthy cells along with the cancer cells in the elimination process [2].

Currently, use of lasers as a surgical device or endoscopic treatment of some diseases such as breast cancer has grown dramatically due to their superiority to current conventional therapies. The main advantages of utilizing lasers include: lack of contact with the hands, reduced bleeding, reduced swelling of the tissue that is happened because of cancer treatment such as radiotherapy and surgery, decreased postoperative pain, disinfection, and less risk of metastasis [3].

Laser radiation has been successful in treating cancer due to its unique properties, such as laser beam coherence, which created a narrow, high-intensity light to hit the target [4]. Laser therapy is based on the target tissue and laser interaction which depends on the irradiation time, laser wavelength, power and energy density. Laser radiation-tissue interactions can be classified into the two categories of photochemical and photothermal interactions. In this study, we investigated the laser radiation effects on breast cancer and healthy cells. Thermal effects can be generated by pulsed lasers or continuous wavelengths lasers, and refer to all the effects that increase local temperature [5].

Various types of laser are used for breast cancer treatments. One type of these lasers is known as photodynamic therapy (PDT) in which photosensitive materials are used as therapeutic agents. These materials produce radical oxygen when laser is irradiated on the cancer cells which is toxic for neoplastic cells [6]. This therapeutic method has minimal invasive damage on the healthy cells [7], [8]. In the variety of studies for cancer treatments, other therapeutic and helpful agents were used along with the PDT to enhance the treatment efficiency. For example, Wang et al. used dendrimers as an effective nanoparticle in breast cancer treatment by using laser radiation [9]. In another study, Lio et al. used Aleo Emodin (AE) as a photosensitive material in the photodynamic therapy to eliminate the KB cells [10]. This photosensitive material is also used in the breast cancer treatment [11]. Photodynamic therapy is also used in *in vitro* studies. For instance, in a study, Alemany-Ribes et al. used laser diode 890 nm wavelength to illustrate the amount of oxygen produced in combination with breast cancer cells were cultured in 2D and 3D conditions which resulted in apoptosis, as well as the rate of destruction of DNA in cancerous tissues, was investigated [12]. In this *in vitro* study, laser diode of 808 nm was used as a laser in photodynamic therapy to investigate its effect on the

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destruction of breast cancer cells.

Another type of laser which is used in breast cancer treatment is Photothermal Therapy (PTT). Thermal treatment causes necrosis in the cells, resulting in fracture of the cell membrane and excretion of digestive enzymes [13]. In the study, Cantu et al. used conductive polymer-based nanoparticles as a therapeutic agent to evaluate the effect of laser on the photothermal damage of MDA-MB-231 breast cancer cells, the compatibility and ability of these nanoparticles to completely eliminate cancerous mass after the use of a laser radiation with a wavelength of 808 nm was observed [14]. In another study, fluorescent microparticles, accompanied by Fluorescein Isothiocyanate (FITC) labeled MCF-7 cells, were incubated and exposed to the laser radiation. Observations have shown that microparticles have entered the cells and stored in the cytoplasm and cause thermal destruction of tissue [15].

Laser induced interstitial thermotherapy (LITT) is another kinds of laser for breast cancer treatment which sometimes is called focal laser treatment. The temperature is locally increased by using this laser so that the cancerous cells in that area die [16]. The use of high-intensity focal laser and low frequencies in the range of 0.8-3.5 MHz increases energy in the cancerous area, and the cells in that area are necrosis and bubble formation, which causes carcinogenic tissue damage [17]. LITT is a standard method in which optical fibers are used to lead the laser light directly toward the target and relieve breast, prostate, gastrointestinal, liver and central glandular tumors [18].

The purpose of this study was to quantify the effect of laser radiation on the destruction of cancer cell clusters so that laser treatment with other therapeutic methods could be more aware and minimize side effects and found new methods. For this purpose, laser diode of 808 nm radiation has been used as a therapeutic agent. In this project, the healthy cell clusters (normal cells of the breast) and cancer cell clusters (breast cancer cells), which are separately placed on the scaffold, are simultaneously exposed to laser radiation, and the results are examined. In the previous studies, most studies and research were focused on the use of lasers in the elimination of cancerous glands, on single cells or *in vivo*, or on cells cultured in the plate. However, the aim of this study was to determine the effect of laser heat on the cluster of cells that have grown on the surface of the scaffold.

II. MATERIALS AND METHODS

A. Chemicals

MCF-7 and MCF-10A cell lines were presented by the Motamed Cancer Institute, Breast Cancer Research Center, Tehran, Iran. PCL with average number molecular weight of 80,000, horse serum, and MTT color were the product of Sigma-Aldrich (St. Louis, USA). Ascorbic acid, chloroform, Glutaraldehyde and dimethyl sulfoxide solution (DMSO) were a product of Merck (Kenilworth, New Jersey, USA).

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS) and

trypsin-EDTA were purchased from Gibco Biocult (Paisley, Scotland, UK). Poison cholera, epidermal growth factor (EGF), Hydrocortisone and insulin were purchased from Iranian Biological Resource Center, Tehran, Iran, and glycine was purchased from Titrachem, Tehran, Iran.

B. PCL Electrospinning

PCL was dissolved in acetic acid and chloroform (3:2) to form a 15% w/w solution. The polymer solution was transferred to a 1 mL syringe connected to a 21G blunt needle (BD precision guide), which was also the positive electrode. The polymer was dispensed using a syringe pump (KD Scientific) at a constant flow rate of 20 $\mu\text{L}/\text{min}$ in a humidity controlled chamber. A rotating mandrel was set at a distance of 10 cm from the tip of the needle and turned at 800 rpm for collecting fibers. Electrospinning of the polymer was carried out by applying a positive voltage of 10 kV rotating collector.

C. PCL Electrospun Scaffold Cross-Linking

Extracellular matrix for cancer cells has a higher rigidity than conventional ECM cells [19]. Therefore, we need a higher stiffness to look like an extracellular matrix of cancerous tissue. Assuming that the cross-linking of the electrospun scaffold could set the mechanical properties of the scaffold to the optimum level, the PCL scaffold, with a 25% glutaraldehyde vapor, was cross-linked for 24 hours. The purpose of the cross-linking of PCL scaffolds was not to optimize the mechanical properties of the scaffold for the growth of cancer cells. Cross-sectional connection was used merely to report the mechanical properties of the scaffold. On the other hand, mechanical properties can influence the results of this study, but they were not the aim of this study.

D. MTT Assay for Evaluating Cells Viability on the PCL Scaffold

First, the MCF-7 cells were placed in 96-well cell culture plate within a 100 μL cell culture medium, and each well usually has between 5 and 10,000 cells. After one day, the cells stick to the bottom of the plate. 22 μL of MTT, a tetrazolium-based color, was added to each well to interact with the mitochondria enzyme in the living cells (about 3 to 4 hours for the oxidation reaction-time reduction). The color of the MTT is yellow in the beginning. After incubation for 48 hours in 96-well plate containing MCF-7 cells and MTT solution in an incubator at 37 $^{\circ}\text{C}$, 100 μL of scaffold extract with 100 μL of healthy and intact culture in each well was deposited from a plate containing cell and MTT solution. It is repeated three times. If the cells are alive, their mitochondria are active and interact with the tetrazolium color, converting into insoluble crystals and purgative forms. This absorbent crystalline solution should be converted to a solution to absorb the absorbance of the ELISA reader. As a result, DMSO was read and absorbed at 570 nm, which is the absorption wavelength for purple. A control specimen was added containing 200 μL cell culture medium, and MTT was retested, and the absorbance Read at 570 nm.

E. Tensile Test from Electrospun Scaffold

For tensile testing, three samples of the cross-linked PCL scaffold at the specified dimensions were prepared. Then, the specimens were pulled at a force of 50 N at a rate of 5 mm/min. The test was done triplicate.

F. Scaffold Degradation Test

In order to investigate the rate of scaffold degradation, three specimens with a specified initial weight were placed inside the 24-house plate. Then, each sample was immersed into the one mL of PBS, and each month samples were weighted with a precision scale of 4 decimal places, and Weight loss percentage-time graph was drawn. The corresponding chart is shown in the results.

G. Cell Culture

The human breast cancer cell line MCF-7 was cultured in a T75 flask in a medium containing DMEM, 10% FBS, 10 µg/mL insulin and 100 U/mL penicillin at 37 °C with 5% CO₂ and 95% air and total humidity. Normal breast cells line MCF-10A were cultured in a T75 flask in a medium containing DMEM: Ham's F12, 5% horse serum, 2 mM L-glutamine, 10 µg/ml insulin, 0.5 µg/ml hydrocortisone, 20 µg/ml epithelial growth factor and 100 ng/ml cholera toxin. They were detached using 0.25% trypsin-EDTA enzyme after they attained 80% confluency and counted by trypan blue and hemocytometer method. The PCL scaffold was placed in ethanol for 20 minutes and then exposed to UV radiation for 20 minutes to sterilize. MCF-7 and MCF-10A cells were seeded on a fiber mat at a density of 1.3×10^4 per Cm² and cultured in their specific culture medium and within a 24-well plate for 5 days. These SEM pictures were taken 5 days after cells seeded onto scaffolds, upon fixing the samples with 3% glutaraldehyde (GA) for 4 h [20]. In order to dehydrate the samples, the scaffolds containing the cell were placed in the alcohol 50%, 60%, 70%, 80%, 90%, 100%, respectively, for 20 minutes, and finally all the samples were distilled in water and one night Under the hood was placed to dry. Eventually, for the SEM imaging, a thin layer of gold was covered on the samples, and eventually, the imaging took place.

H. Laser Radiation on The Mcf-7 And Mcf-10A Cell Clusters Were Cultured on the PCL Scaffold

Laser diode of 808 nm was used to radiate on the cells which were cultured on the scaffold through the fiber. The laser was radiated in the various period and powers (Table I). Immediately after a laser radiation, a viability test was performed. To perform this test, the medium was first removed from the scaffold surface in a 24-well plate, and the samples were transferred to a new plate. The full scaffold was not completely fill each well so that the cells may stick to the bottom of the plate, which can make errors in the MTT test results. 500 µL cell culture medium was poured onto the scaffold surface, and 55 µL of MTT color was added to the

medium. The cells were incubated for 4 hours at 37 °C with 5% CO₂, 95% air and complete humidity. The medium was replaced with 500 µL DMSO, and cells were incubated for 30 minutes at room temperature. Finally, with the help of Eliza reader (Biotek), the optical density (OD) was determined. Powers were selected based on an article [21].

TABLE I
 TIME & POWER APPLIED TO THE CELLS CULTURED ON THE PCL SCAFFOLD

Power(W)	Time 1 (min)	Time 2 (min)
1 W	1	5
2 W	1	5

III. RESULTS

A. Investigating and Measuring the Optimal Criteria for the Construction of PCL Scaffold

20 samples were prepared by means of electrospinning, one samples with the appropriate morphology (fiber diameter, pore size and density, no beads) was selected to find the percentage of porosity with suitable fiber diameter and other characteristics for the final scaffold.

The size of the porosity is significant in choosing the final parameter for the scaffold. MCF-7 cells have high adhesion properties to PCL scaffolds and can grow in porosity. Therefore, porosity percentage, size and also the fiber diameter are very important. Sample which has an average porosity of 2.387 µm with a porosity of 40% was selected as a final scaffold.

B. Morphology and Microstructure

Fig. 1 shows SEM micrographs of electrospun PCL. There are many variables effecting on the fiber diameters, including electrospinning parameters, polymers properties, solvent system and its properties [22]. As seen in Fig. 1, the diameter of the fibers was uniform, and the beads were not visible inside the scaffold. There is also no rupture in fibers, which contributes to the uniformity and decrease of mechanical properties of the scaffold.

C. MTT Test Results of Electrospun Scaffolds for Mcf-7 Cancer Cells

Generally, cells, such as cancer cells, need a proper substrate for growth and pluripotency. Therefore, after constructing PCL scaffolds, a scaffold viability test was taken in the presence of breast cancer cells with MCF-7 cell line and the chart and data were drawn by EXCEL software, as shown in Fig. 2.

According to the survival test results of the scaffold, with a viability percentage of 91.3%, this scaffold is a suitable substrate for growth and proliferation of these cells and produces very low toxicity. PCL polymer has very good biocompatibility [23]-[27] which is evident in the results, and applied in the field of tissue engineering or tissue regeneration as scaffold materials.

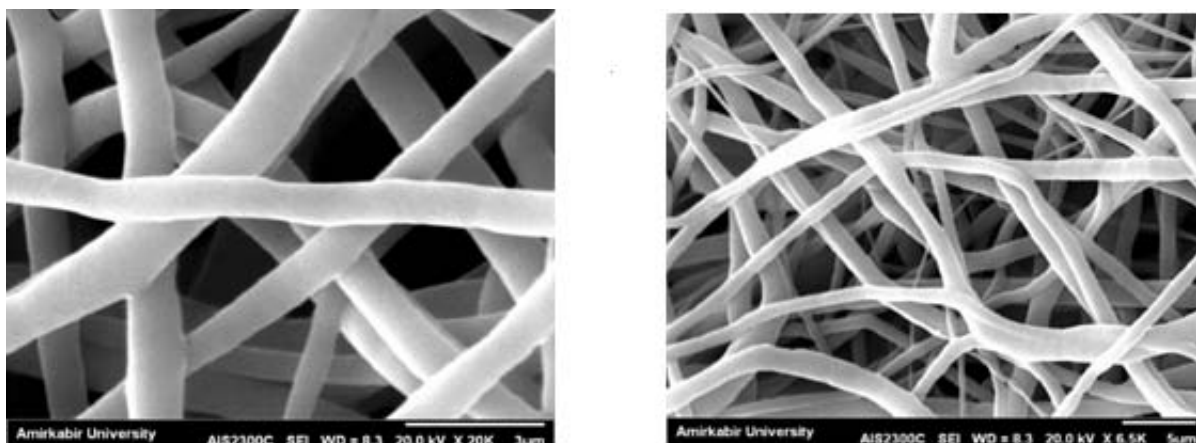


Fig. 1 SEM images of PCL electrospun fibers with a magnification of 20 K, average fiber diameter = 900 nm, voltage 10 kV, nozzle to collector distance= 10 Cm, the solution from the nozzle exit rate of 0.2 ml/min were, magnitude: 20 K and 6.5 K

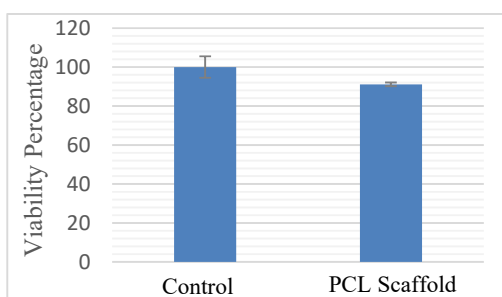


Fig. 2 MTT test results for PCL scaffold for MCF-7 cells. Viability percentage: control= 100%±/-5.52%, PCL scaffold= 91.3%±/-0.98%

D. Degradation Test

To assess the PCL scaffold biodegradability, the scaffold was placed within PBS for six months, and the scaffold weight loss was measured using weight balance. Using the EXCEL software, the corresponding diagram was drawn and shown in Fig. 3.

As shown in Fig. 2, the rate of PCL degradation is meager, and from month four the speed of weight loss increases slightly, and PCL needs more than two years for degradation [28]. Each time MCF-7 and MCF-10A cells are cultured for a maximum of one week so that no damage occurs.

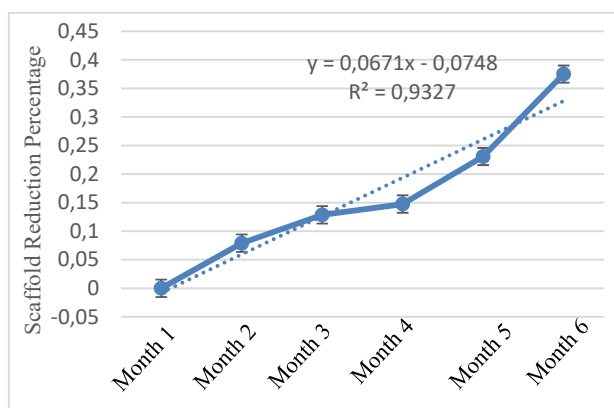


Fig. 3 The PCL scaffold degradation test charts, for six months, the rate of increase in weight loss percentage = 0.07 / month

E. Mechanical Test

The mechanical properties of electrospun scaffold PCL Test results are shown in Fig. 4. The mechanical properties of the scaffold were plotted in the conditions of cross-linked electrospun fibers.

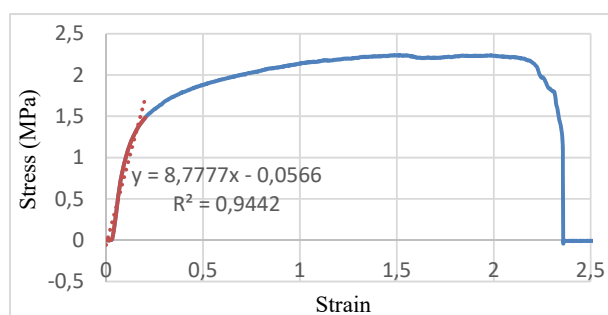


Fig. 4 Stress-Strain Diagram, with cross-linking. Young's modulus= 8.8 +/- 0.6 MPa, ϵ^{ult} = 2.23 +/- 0.022, strain at break= 200%

Cancer cells can grow, proliferate, and migrate on surfaces of greater stiffness [19]. The mainstay of the growth of healthy and cancerous cells in this study was the PCL-provided crosslinking fibers, and its mechanical properties were reported as a constant parameter.

F. Morphology of MCF-10a Breast Cells on the Scaffold

In order to compare morphology and finally to compare the effect of laser on the destruction of healthy and cancerous cells, normal cells were grown on the scaffold surface, SEM images were taken, as shown in Fig. 5. With the help of ImageJ software, the average size of the cell size was measured on the scaffold surface.

G. Morphology of MCF-7 Breast Cancer Cells on the Scaffold

MCF-7 breast cancer cells were cultured on a scaffold surface as described in the methods part, and the images were taken with SEM and shown in Fig. 6. The average cell cluster size was measured on the surface of the scaffold using ImageJ software.

As shown in Figs. 2 and 3, cancerous cells are adhering and spreading more than healthy cells on the surface of the PCL scaffold. The size of the cancerous cells spread on the scaffold surface is about 98.120 microns, while healthy breast cells

spread over the surface of the scaffold with an average size of 28.36 microns. As a result, cancer cells have higher adhesion sites than normal cells. Also, the growth and expansion rates of cancer cells are much higher compared to healthy cells.

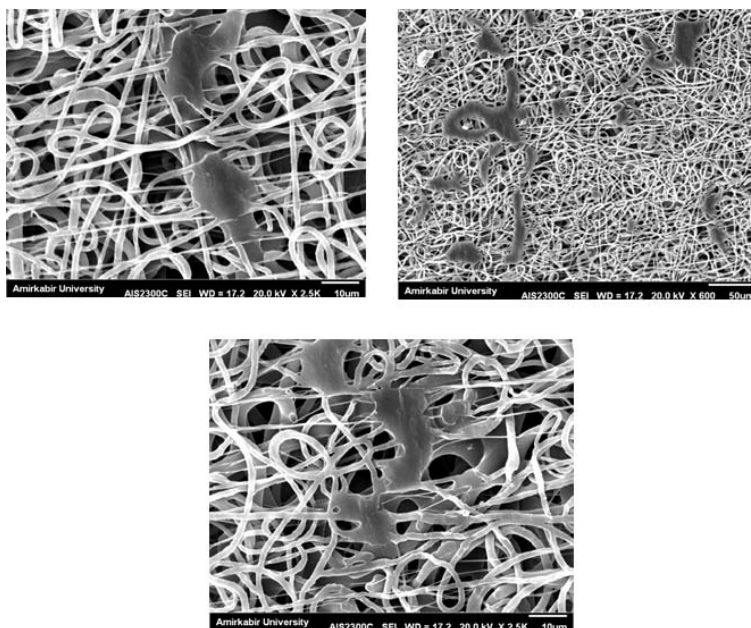


Fig. 5 SEM images of MCF-10A cells cultured on PCL scaffold surface, after five days in DMEM medium, the average cell size on the scaffold surface is 28.36 µm. Magnitude: 600, 2.5K

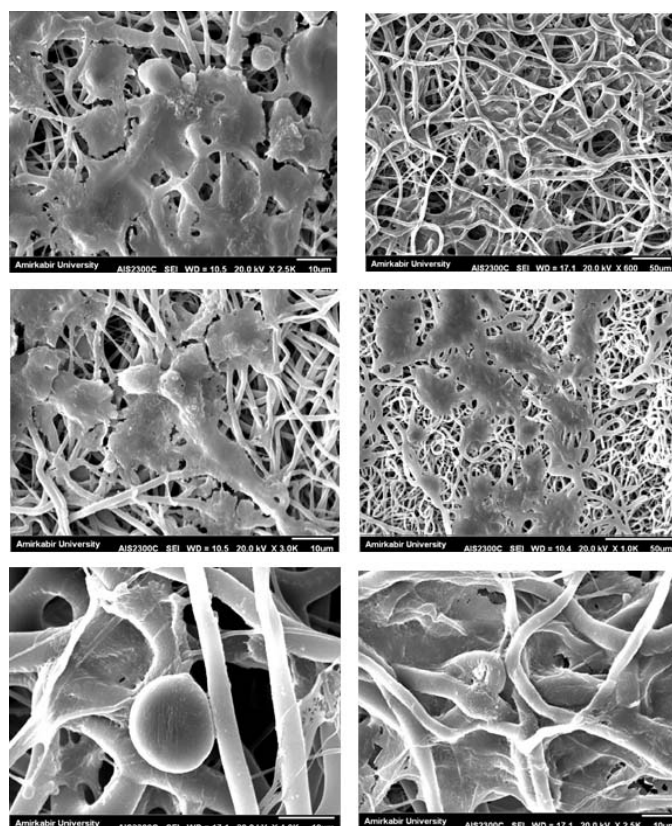


Fig. 6 SEM images of MCF-7 cells cultured on PCL scaffold surface, after five days in DMEM medium, the average size of cells on the scaffold surface is 98.120 µm. Magnitude: 600, 1K, 2.5K, 3K, 4K

H. Laser Diode 808nm Radiation Effect on Cells

After laser diode of 808 nm radiation, viability of MCF-7 breast cancer cells and MCF-10A breast cells, cultured on the PCL scaffold, was obtained by MTT testing, which is shown in the following diagrams. Figs. 7 and 8 are plotted using Excel software.

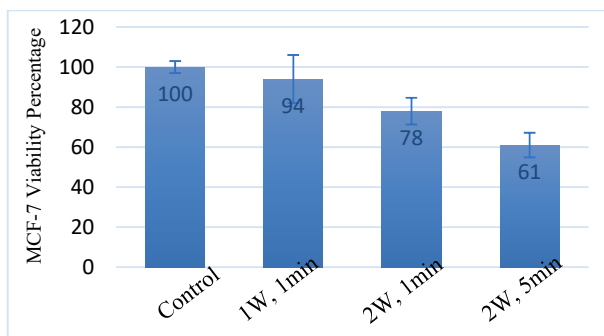


Fig. 7 Percentage of viability MCF-7 cells grown on the surface of electrospun PCL scaffold after laser radiation at different powers and times

According to Fig. 5, the duration of one minute for laser radiation did not significantly affect the survival of MCF-7 cells. By increasing the length of radiation and increasing the amount of active power, the number of live cells has decreased. The ultimate power diode laser 808 nm 2 W, which was radiated on cells cultured on the scaffold PCL for 1 and 5 min. Most of the cancer cells died after the laser radiation in the order of 5 minutes in the 2W power.

As shown in Figs. 5 and 6, a laser with a power of 1 and 2 W is radiated into healthy breast cells with MCF-10A cell line. MCF-7 and MCF-10A cells are exposed under laser radiation beneath the biological hood under both the same temperature and physical conditions. The results of the viability of healthy cells after the laser radiation showed that the power of 1W and the 1min laser light exposure time on cell-based scaffolds did not play a significant role in reducing cell survival. The power of 2W throughout 1.5 min decreased the health of healthy cells to some extent.

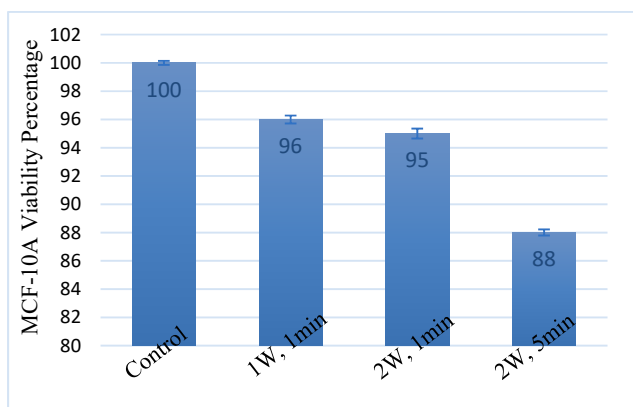


Fig. 8 Percentage of viability MCF-10A cells grown on the surface of electrospun PCL scaffold after laser radiation at different powers and times

Figs. 4 and 5 shows that, in general, MCF-10A cells exhibit higher resistance to laser radiation compared to MCF-7 cells. As can be seen, there was no significant difference in the survival of MCF-10A and MCF-7 cells after laser irradiation in 1 min and 1W. In comparison, the cell survival rate is reduced by applying 1W and 5 min radiation. It can be concluded that the time and power are effective parameters in eliminating cells. At 2 W and 5 min the loss of healthy cells can be neglected, and this represents a survival of over 80% of healthy cells. While at 5min, the survival rate of cancer cells was 61%.

In general, there are three types of cell death including apoptosis, necrosis, and autophagy. Apoptosis is a planned cell death during which the cell membrane is fragmented, or blisters are formed on the cell membrane [29]. During necrosis mechanism cells death occurs due to the external agents such as poison electrical and chemical signals, heat [30]. Finally, cells can be killed by Autophagy mechanism, a natural mechanism of cell defense. When the food does not reach the cell sufficiently or if the cell must destroy aggressive agents such as bacteria and viruses, autophagy is necessary to eliminate waste and reduce cell consumption. Also, the cell destroys waste materials that they produce or aging components thus destroy and use their raw materials to produce new components and materials [31].

Given that laser radiation on the cells is an external agent and is not the expected death of cells, it can be concluded to some extent that cells have been destroyed through the laser light through the mechanism of necrosis.

In the body, cancer cells are much more fragile than healthy cells due to lack of oxygen, acid pH, and heat [32]. This study has been conducted in the *in vitro* environment in which cell viability has decreased to 61%. According to the results of this study, the lack of oxygen and the acidity of the environment inside the body in cancerous areas is not the only reason for the disappearance of cancer cells after a laser collision.

IV. CONCLUSION

In conclusion, it can be stated that with the help of a laser alone, the cell survival rate eventually decreased by up to 61%. Still, 30% of the cells are still alive. To increase the efficacy of treatment, light-sensitive substances or chemotherapy drugs with laser radiation in the future has been used but the results of this study made it much easier to find the right medicine.

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