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COMBINED EFFECTS OF CHEMOTHERAPY AND INDOCYANINE GREEN MEDIATED PHOTODYNAMIC THERAPY ON EX *VIVO* HUMAN PRIMER BREAST CANCER CELLS

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MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

İSTANBUL, FEBRUARY / 2014

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T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

İndosiyanin yeşil molekülünün *ex-vivo* insan primer meme kanseri hücreleri üzerindeki Fotodinamik Terapi ve birleştirilmiş Kemoterapi etkisinin araştırılması

AYŞENUR KİRİŞ

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Ayşenur KİRİŞ, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID 52011121, successfully defended the **thesis/dissertation** entitled "**Combined effects of Chemotherapy and indocyanine green mediated Photodynamic Therapy on ex *vivo* human primer breast cancer cells**", which he/she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To dear my family who support and love me all time,

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LIST OF SYMBOLS

®	Registered trademark symbol
μ	Micro
ν	Frequency
l	Liter
M	Molar

ABBREVIATIONS

CAM : Complete Assay Medium
cm : Centimeter
DMEM : Dulbecco's Modified Eagle Medium
Hp : Hematoporphyrin
Hr : Hour
ICG : Indiocynanine Green
IST : Istanbul
J : Joule
kg : Kilogram
LED : Light Emitting Diodes
mg : Milligram
min : Minute
mm : Millimeter
nm : Nanometer
O_2 : Oxygen
-OH : Hydroxyl Radical
PBS : Phosphate Buffered Saline
PDT : Photodynamic Therapy
PS : Photosensitizer
PSs : Photosensitizers
ROS : Reactive Oxygen Species
TDC : Test Drug Concentration

TR : Turkey

UK : United Kingdom

USA : United States of America

US-FDA : United States Food and Drug Administration

μM : Micromolar

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SUMMARY

COMBINED EFFECTS OF CHEMOTHERAPY AND INDOCYANINE GREEN MEDIATED PHOTODYNAMIC THERAPY ON EX *VIVO* HUMAN PRıMER BREAST CANCER CELLS

Ayşenur KİRİŞ

Biomedical Engineering Programme

MSc Thesis

Advisor: Assist. Prof. Dr. Haşim Özgür TABAKOĞLU

Breast cancer is most common and lethal cancer type among women. According to WHO estimates in 2013 of 234,580 men and women are expected to be diagnosed with breast cancer. Aim of this study investigation of Photodynamic therapy (PDT) synergetic chemotherapy and alone PDT effects of ICG on ex vivo primer human breast cancer cells.

Primer breast cancers cells obtained from Bezmialem Foundation University Medicine Faculty Hospital. The photosensitizer ICG (50 μM) was applied to primer breast cancer cells in combination with laser irradiation (780 nm) exposure for 35 min and then incubated for 24 h. After 24 h chemotherapeutic agents (paclitaxel, doxorubicin, 5fluorouracil) applied on combine therapy groups. Cell viability was analyzed with ATP-TCA assay.

According to cell viability assay results, ICG is a effective photosensitizer for primer breast cancer cells. In combined PDT and chemotherapy groups synergetic effect was observed and viability decreased in significantly.

Keywords: Photodynamic Therapy, Chemotherapy, ICG, ATP-TCA, LED.

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ÖZET

İNDOSİYANİN YEŞİL MOLEKÜLÜNÜN *EX-VİVO* İNSAN PRİMER MEME KANSERİ HÜCRELERİ ÜZERİNDEKİ FOTODİNAMİK TERAPİ VE BİRLEŞTİRİLMİŞ KEMOTERAPİ ETKİSİNİN ARAŞTIRILMASI

Ayşenur KİRİŞ

Biyomedikal Mühendisliği Programı
Yüksek Lisans Tezi

Danışman: Yrd. Doç.Dr. Haşim Özgür TABAKOĞLU

Meme kanseri kadınlarda en sık görülen ve en çok ölüme neden olan kanser tipidir. 2013 yılı WHO tahminlerine göre 234.580 erkek ve kadına meme kanseri tanısı konması beklenmektedir. Bu çalışmada indosiyanin yeşil molekülünün *ex-vivo* insan primer meme kanseri hücreleri üzerindeki fotodinamik terapi (FDT) ve FDT ile birleştirilmiş kemoterapi etkisi araştırılmıştır. Primer meme kanseri hücreleri Bezmialem Vakıf Üniversitesi Tıp Fakültesi Hastanesi'nden elde edilmiştir.

Primer meme kanseri hücreleri üzerine 50 μM konsantrasyonda ICG eklenmiş ve inkübe edilmiştir. 24 saat inkübasyon sonrasında 780 nm LED sistemiyle 35 dakika uyarılmıştır. 24 saat sonra birleştirilmiş tedavi gruplarına 100 TDC paklitaksel, doksorubisin ve 5fluorourasil uygulanmıştır. Hücresel canlılık analizi için 24 saat sonra ATP-TCA canlılık testi uygulanmıştır.

Canlılık testi sonuçlarına göre ICG kullanılarak yapılan FDT primer meme kanseri hücreleri üzerinde etkili olmuştur. FDT ile birleştirilmiş kemoterapi kanserli hücre yaşamsallığını anlamlı bir şekilde düşürmüştür.

Anahtar kelimeler: Fotodinamik Terapi, Kemoterapi, ICG, ATP-TCA, LED.

CHAPTER 1

1.1 Purpose of the Thesis

Photodynamic therapy (PDT) is a clinically approved cancer therapy methods. PDT application has destructive effect on cancer cells. PDT has many advantages over traditional methods like chemotherapy, surgery, radiotherapy. These advantages can be listed as; minimal side effect, lack of drug resistance mechanisms, less scarring, less invasive, can be targeted and unlike radiation, can be repeated several period.

The purpose of the study is investigation of *ex-vivo* photodynamic therapy application with indocyanine green (ICG) molecules on primer breast tumors. In addition, combined effects of PDT and chemotherapy will be examined with three different chemotherapeutic agents namely, paclitaxel, doxorubicin and fluorouracil.

1.2 Motivation

Cancer is a major health problem for humanity. Cancer survival is increasing in Europe with the early diagnosis and reduced success of treatment [1]. According to the National Center for Health Statistics data 1,660,290 people in United States get a cancer disease, 580.350 people are expected to die because of cancer. 40,030 deaths are expected from 234.580 new breast cancer cases in both genders [2].

The recent research has provided a lot of new information about tumor biology and lead to design new cancer therapy drugs [3]. Despite progress, with few exceptions, there have been slight differences in the treatment of cancer. These situations emphasize the need to focus on new approaches for treatment of cancer [4]. Photodynamic therapy is a minimally invasive therapeutic procedure and approved by US-FDA. PDT's main

reaction is the production of singlet oxygen species by using light source irradiation of a photosensitizers accumulated in tumor cells [5]. PDT has advantages over the other treatment methods. These are; being minimally invasive, targeted tumor cell destruction without causing loss of healthy tissue, repeatable, good clinical output and cost effective [4-6]. On the other hand there is insufficient data for clinical application yet.

Challenge in PDT is to design new photosensitizers suitable deeper tumors. The photosensitizer indocyanine green (ICG) has maximum absorption at around 805 nm which can penetrate deeper into tissue. For this reason ICG-mediated and chemotherapy combined PDT on primer breast cancer cells were tested with ATP-TCA cell viability assay.

CHAPTER 2

2.1 Cancer

In today's world, one of the most important healthy problems is cancer [1, 2, 7-11]. Cancer is a group of diseases and defined by unrestrained cell growth and diffuse of abnormal cells. In the cases of the uncontrollable spread, it can result in death. Cancer is caused by extrinsic and intrinsic reasons. These causal factors may be active together or separately.

Cancerous cells are also called malignant tumors. A tumor is formed by an abnormal growth of cells. There are three different tumor types; benign, pre-malignant or malignant whereas cancer is by definition malignant [12-14].

Breast cancer usually doen't show any symptoms when the tumor is small and treatable [15-17]. Bigger tumors can be a painless mass. Common symptoms are changes to the breast properties: thickening, swelling, distortion, tenderness, skin irritation, redness, scaliness, nipple abnormalities, ulceration, retraction and spontaneous discharge. Normally, bening tumors lead to breast pain and is not an early sign of breast cancer.

2.2 Cancer Therapies

The most effective method in the cancer treatment is early detection. With early diagnosis many of the cancers have been treated. According to type, pathology, histochemistry of cancer, account tumor size, extent of spread, and other characteristics, as well as patient preference treatment options have been variable. In the cancer therapy, single or multiple treatment procedure can be applied at the same time.

Surgery is most preferred procedure using to remove solid tumor tissues. Other most commonly used treatment methods are radiation therapy, chemotherapy, hormone therapy or targeted therapy [1, 2, 18].

Chemotherapy is another procedure which consists of anticancer drugs. Traditional chemotherapeutic drugs act by destroying cells that divide rapidly which is one of the main properties of most cancer cells [18-19]. Chemotherapy has lots of side effects that depend on the type of drugs. Some of the side effects are immunosuppression and myelosuppression, typhlitis, gastrointestinal distress, anemia, fatigue, nausea and vomiting, hair loss, secondary neoplasm, infertility, teratogenicity, neurological adverse effects and organ damage. Therefore, to develop a new treatment has a vital importance.

Radiation therapy is the medical term, using ionizing radiation for cancer treatment to control or to destroy malignant tumors. Ionizing radiation causes the cellular death by damaging the DNA [18, 20]. Besides, accordingly chemotherapy, radiotherapy has a number of acute and late side effects.

Except traditional methods, there is some alternative cancer therapy methods; hormonal, bone marrow transplantation, stem cell therapy and photodynamic therapy [18, 21].

Photodynamic therapy is approved cancer therapy procedure, that can involves administration of a photosensitizer following irradiation of the sensitizer by using light source which has a specific wavelength [22, 23, 24]. As a result of PDT, in the presence of oxygen, photochemical and photobiological process lead to irreversible photochemichal damage to tumor tissue.

2.3 Photodynamic Therapy

Light has been used for diagnosis and treatment more than thousand years. Sun was used for light therapeutic effect for treatment to vitiligo, psoriasis, and psychosis in Egypt, India and Chine [25].

First studies about photodynamic therapy were begun in Europe in last century. Physician Niels Finsen treated cutaneous tuberculosis with ultraviolet light and smallpox using red light by generated by an arc lamp. Nilsen was awarded Nobel Prize with his PDT study [26]. Oscar Raab was shown specific wavelengths of light were

lethal to the organism paramecia irradiated to the chemical material acridine in 1900 [27].

Photosensitivity reactions described by Hausmann in mice that had been administered haematoporphyrin in 1911 [28]. German Friedrich Meyer-Betz in 1913, was showed haematoporphyrin parallel effects could be induced in humans by injected himself with 200 mg haematoporphyrin. Frenchman Policard was described localization of the porphyrins in to malignant tissue in 1924 [29-32].

Lipson and Baldes was studied accumulation of hematoporyrin derivative (HpD) on the tumor in 1960. This HpD prepared by Dr. Samual Schwartz and its use in the photodetection of tumors. FDA approved the first photosensitizing drug the Photofrin® (PH) in 1987 [33]. In the current situation, there are a number of photosensitiziers approved by FDA for cancer therapies [33-36].

Photodynamic effect was a discovery in 1900s [37]. Since discovery of PDT, studies have been focused on mechanism of action behind this theraphy method [38-40]. Clinically based applications with different photosensitisers have been studied [33]. Three basic components are needed for PDT reaction to occur; a photosensitizer, singlet oxygen and a light source (Figure 2.1).

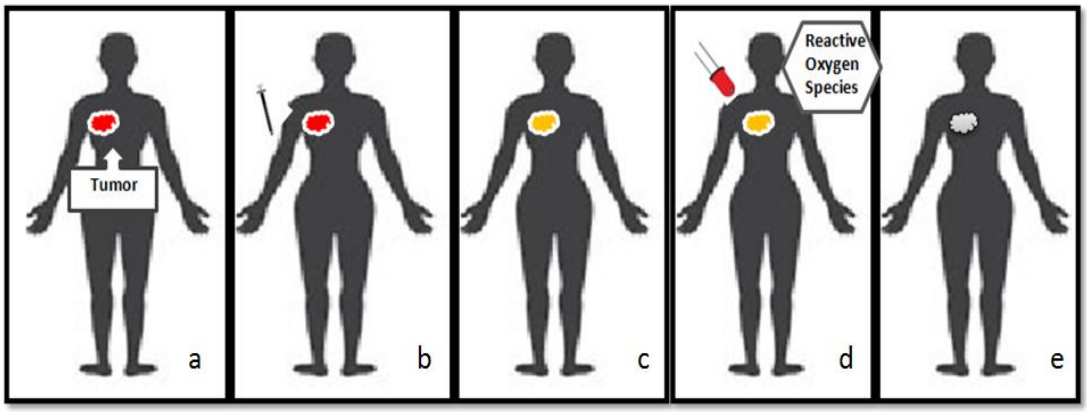

Figure 2.1 Photodynamic therapy procedure. Firstly the selective administration and accumulation of a photosensitizer in a cancer tissue. After than exposure with light of a particular wavelength, thereby initiating tumor necrosis probably through formation of reactive oxygen species.

2.3.1 Photochemistry and Photophysics of Photodynamic Therapy

Light, photosensitizer and oxygen are the main mechanism constituents of photodynamic reaction (Figure 2.2). Generally two different pathways occur depending on the existence of singlet oxygen. These pathways are called Type 1 and type 2 shown in Figure 2.3 [41-44].

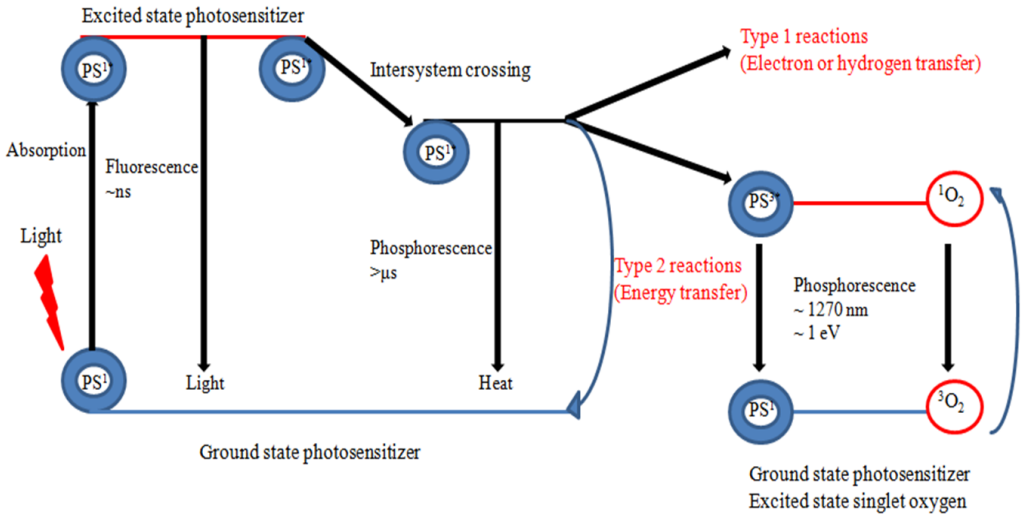

Figure 2.2 Photosensitization Processes Pictured by a Modified Jablonski Diagram

In a Type 1 reaction, PS can react with a substrate. After than a proton or an electron transfer can occur to form a radical anion or cation. These radicals can be supportive to react with oxygen to generation of reactive oxygen species (ROS) [45-47]. On the other hand in a Type 2 reaction, the triplet PS can transfer its energy directly to molecular oxygen to form excited state singlet oxygen. Depend on the PS properties, concentrations of substrate and oxygen; these reactions can be occurred simultaneously or at a uncertain extend which are still remain under investigation [48-50].

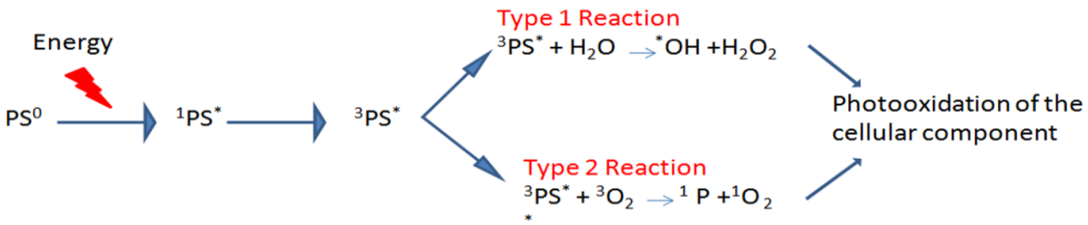

Figure 2.3 The basic photosensitized oxidation process

PDT can lead to 3 main cell death morphotypes [50, 51]. These are apoptosis, necrosis and autophagocytosis. Apoptosis is common seen PDT response of cell [50-52].

Apoptosis has some markers. These are chromatin condensation, cleavage of chromosomal DNA into internucleosomal fragments, membrane blebbing, cell shrinkage and the formation of apoptotic bodies without plasma membrane breakdown.

Necrosis is characteristically appear by vacuolization of the cytoplasm, breakdown and swelling of plasma membrane, resulting in an inflammatory reaction depend on the release of cellular contents and proinflammatory molecules. Autophagy is morphologically seen a massive vacuolization of the cytoplasm [50-54].

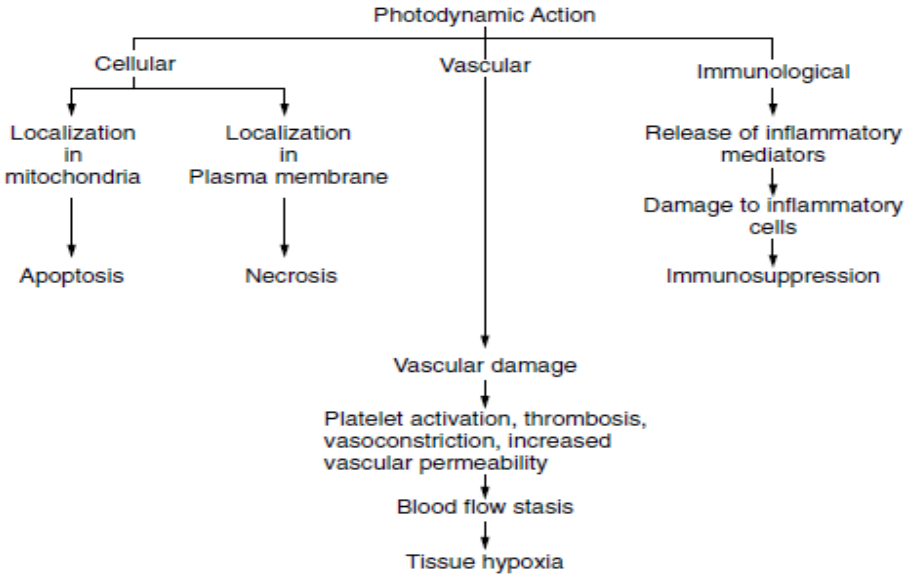

Figure 2.4 Three principal mechanisms of PDT [55]

2.3.2 Photosensitizers

Clinically approved photosensitizers (PSs) can be categorized according to their chemical structure (Table 2.1). Most of the PSs, like protoporphyrin, are composed of a tetrapyrrole backbone (Figure 2.6) [50, 54, 56].

Table 2.1 Classification of PSs [56]

Family Name	Photosensitizers
Porphyrin platfrom	HpD, HpD-based, BPD, ALA, Texaphyrins
Chlorophyll platform	Chlorins, Purpurins, Bacteriochlorins
Dyes	Phtalocyaine, Napthalocyanine

Table 2.2 Photosensitizers and Clinical Applications [54]

PHOTOSENSITIZER	STRUCTURE	WAVELENGTH, nm	APPROVED	TRIALS	CANCER TYPES
Porfimer sodium (Photofrin) (HPD)	Porphyrin	630	Worldwide		Lung, esophagus, bile duct, bladder, brain, ovarian
ALA	Porphyrin precursor	635	Worldwide		Skin, bladder, brain, esophagus
ALA esters	Porphyrin precursor	635	Europe		Skin, bladder
Temoporfin (Foscan) (mTHPC)	Chlorine	652	Europe	United States	Head and neck, lung, brain, skin, bile duct
Verteporfin	Chlorine	690	Worldwide (AMD)	United Kingdom	Ophthalmic, pancreatic, skin
HPPH	Chlorin	665		United States	Head and neck, esophagus, lung
SnEt2 (Purlytin)	Chlorin	660		United States	Skin, breast
Talaporfin (LS11, MACE, NPe6)	Chlorin	660		United States	Liver, colon, brain
Ce6-PVP (Fotolon), Ce6 derivatives (Radachlorin, Photodithazine)	Chlorin	660		Belarus, Russia	Nasopharyngeal, sarcoma, brain
Silicon phthalocyanine (Pc4)	Phthalocyanine	675		United States	Cutaneous T-cell lymphoma
Padoporfin (TOOKAD)	Bacteriochlorin	762		United States	Prostate
Motexafin lutetium (Lutex)	Texaphyrin	732		United States	Breast

The successful PS should be chemically pure drug with preferential uptake in tumor, good stability in storage, rapid clearance, low manufacturing costs and a strong absorption peak between 600-800 nm [57,58].

H0ematoporphyrin is first-generation photosensitizer. Hematoporphyrin's commercial using form is Photofrin® [59-61]. Photofrin® is still the most widely employed PS in clinic but has some disadvantages; a lengthy skin photosensitivity and low absorbance at 630 nm.

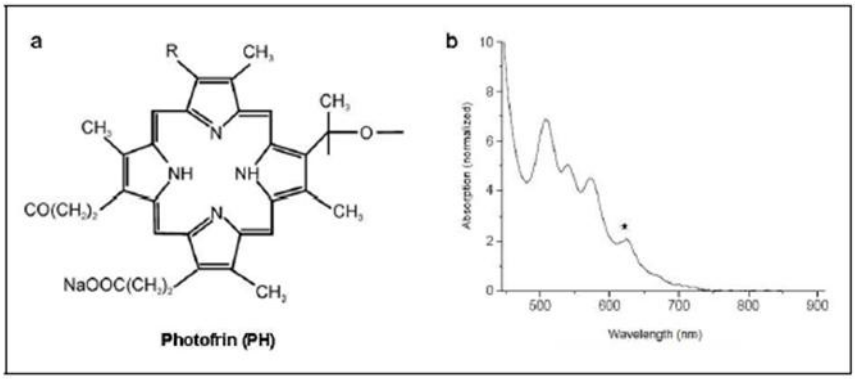

Figure 2.5 Photofrin®'s molecular structure and absorption spectra [56]

Indocyanine green (ICG) is water-soluble, high molecular weight and anionic tricarbocyanine dye [62-65]. ICG has been used for medical diagnosis and approved by FDA in 1956. The absorption spectrum of ICG between 600-900 nm is shown on Figure 2.6. ICG's maximum absorption peak at around 800 nm [62,66]. It is an advantage for

PDT application; due to higher penetration depth of light around these wavelengths deeper tumors can be treated.

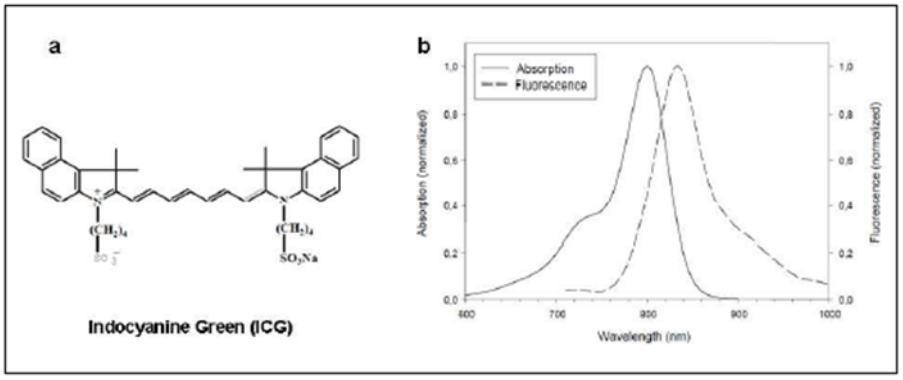

Figure 2.6 ICG's molecular structure and absorption spectrum [67]

In-vitro effects of ICG mediated PDT have been investigated on a variety of human cell lines. Abo Zeid et al showed that viability of MCF-7 cells were not reduced so much when MCF-7 breast cancer cells were treated with only ICG or only laser irradiation [68]. Abdel-Megid Mamoon et al shown ICG mediated PDT induce apoptosis in melanoma cells [69]. In the another study, 3 different pancreatic cancer cell lines (MIA PaCa-2, PANC-1, and BxPC-3) were incubated with ICG and irradiated to infrared light at 0,45 W. Results of the study showed that ICG mediated PDT significantly induce viability of pancreatic cancer cells [70]. According to another study, both ICG-ormosil PEBBLEs and free ICG had similar phototoxic effect on cancer cell lines (MCF-7 and HepG2) [71].

2.3.3 Light Sources

Irradiation with a light source is fundamental step of PDT application. Light can be delivered directly or with optical fibers [72, 73]. The success of PDT depends on combinations of photosensitizers, choice of light source and treatments parameters.

Tissue-light interaction related with measuring tissue absorption and scattering properties. Turbid media has different heterogeneous tissue chormophores. Like hemoglobin, oxyhemoglobin, melanin and water that absorb the light at a specific range (Figure 2,7) [74]. Each choromophore absorbs different wavelength of light. Optical window, also known as therapeutic window, determines maximum depth of penetration

into tissue (Figure 2.8). Optical window of turbid medium is between 600-1300 nm. In this range 850 nm has maximum penetration depth [75].

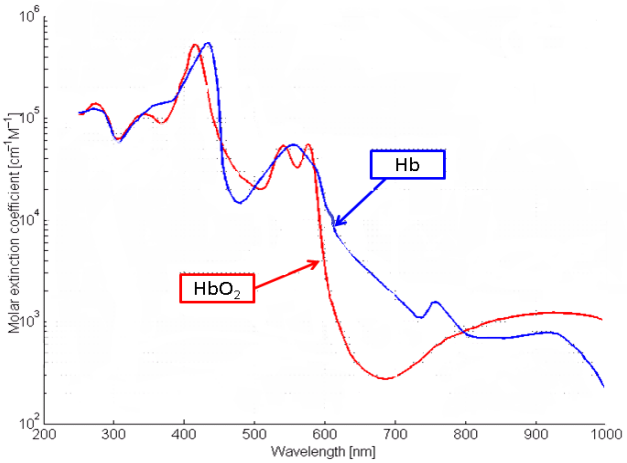

Figure 2.7 The absorption spectra of Hb (hemoglobin) and HbO_2 (oxyhemoglobin) [76]

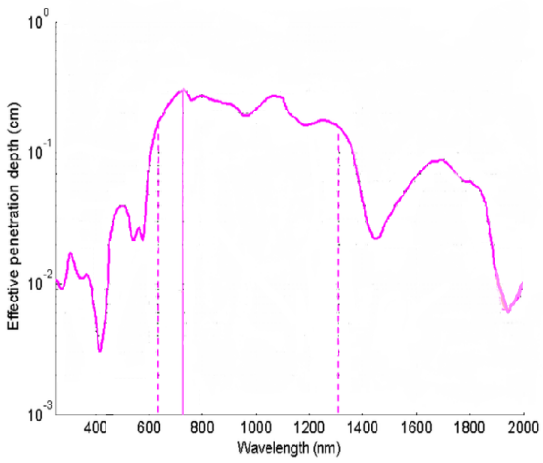

Figure 2.8 Effective penetration depths in breast tissue. Effective attenuation coefficient: λ_{min} = 730 nm; NIR window = (626 - 1316) nm [77].

From the beginning of PDT studies, conventional lamps were used [78]. Lamps generate non-coherent light but with using filters, output of lamps was chosen. Laser and non-laser sources are preferred in PDT studies. Lasers have some advantages. These are; coherent light beam, monochromatic wavelength and easy calculation of dosimetry. Argon lasers and metal vapour lasers are first lasers used in PDT [79]. An optical fiber is necessary for delivered light to PDT application area. Type of optic

fibers can be fabricated appropriate for the target area. However, lasers are expensive and some of them not user friendly.

In recent years LED (light emitted diode) systems have been used in PDT. LEDs based light source have some advantages for clinical and laboratory use. LEDs have a range of wavelength band from 350 nm to1100 nm and can provide the power output up to 250 mW/cm^2 [80]. LEDs have important distinctiveness. These are lower prices than lasers and versatility in function. LEDs can be design and adapted to several target area.

2.4 Clinical Applications of Photodynamic Therapy

Photodynamic therapy is unique anticancer therapy, which requires a accumulation of photosensitizer before light irradiation. Latency time is relatively short when compared to conventional methods. In contrast to PDT, most cancer therapy applications require long term treatments at hospital. Another advantage of PDT is that treatment can be repeatable [81, 82]. In radiotherapy procedure treatment requires 6-7 weeks irradiations and can't be repeatable for several times. Other cancer therapy method; surgery is a complex procedure, performed under with anesthesia and hospital stay during healing period is as long as 6-7 weeks [83].

Chemotherapy is another popular treatment method with using cytotoxic anti-neoplastic drugs. Chemotherapy is mostly applied together with radiotherapy or surgery. In recent years various treatment strategies developed in chemotherapy. These are; combined therapy, combination of chemotherapeutic drugs, intensification chemotherapy, neoadjuvant chemotherapy, adjuvant chemotherapy and palliative chemotherapy [84]. Chemotherapy procedure has different treatment durations depend on patient conditions. In breast cancer treatment doxorubicin, paclitaxel, 5fluorouracil, methotrexate and cyclophosphamide are most popular drugs [85]. Chemotherapeutic drug dosage calculation is difficult. If dose will be low, treatment cannot be successful. If dose will be high, treatment can lead to many of systemic side effect [86]. Chemotherapy has many of systemic side effects. PDT is a local treatment and cannot be lead to many of side effect [87]. Also PDT has low cost effect than other treatment methods.

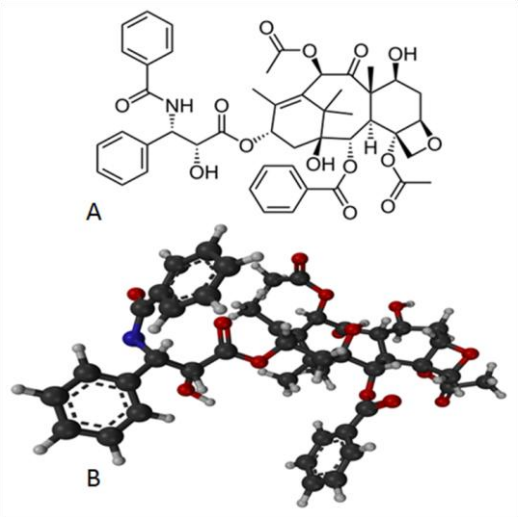

Figure 2.9 Chemical structure and 3D model of Paclitaxel [88]

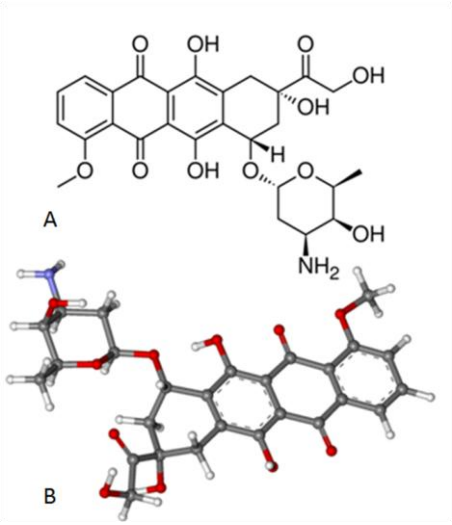

Figure 2.10 Chemical structure and 3D model of Doxorubicin [89]

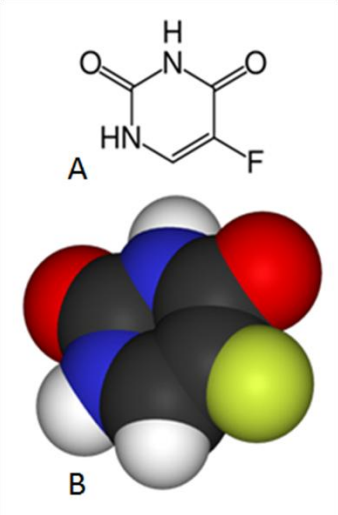

Figure 2.11 Chemical structure and 3D model of 5Fluorouracil [90]

Since first PDT applications, there have been more than 200 clinic studies of PDT. The first clinic application of PDT was applying for bladder cancer with using photofrin in Canada. PDT approved for advanced esophageal cancer in US at 1995. In 1998, Kato et al. showed and proposed PDT treatment with using photofrin for lung cancer [91, 92]. ALA was approved in 2000 for actinic keratosis and basal cell carcinoma. Foscan-PDT was approved in 2001 for head and neck cancer cancers in Europe [82]. Besides, PDT is using in breast, brain, gynecological, urological and gastrological cancers. Many studies showed PDT-combined therapies have great potential to develop to cancer therapy [93].

CHAPTER 3

3.1 Cell Culture Protocol

Ex vivo human primer breast tumors were obtained from Bezmialem University Medicine Faculty General Surgery Department after mastectomy surgery. Ethic committe report obtained from Bezmialem University Clinical Research Ethic Committee (This study was approved by the Bezmialem Foundation University Clinical Research Ethic Committee with 71306642/050-01-04-72 number and date of 25.03.2013)

Tumor tissues transferred with DMEM (DMEM; Gibco BRL-Invitrogen, Holland) which consist of %1 penicillin/streptomycin and %1 HEPES. Transfer medium was removed with a sterile Pasteur pipette connected to a controlled, continuous vacuum pump system and tissue was placed the petri dish with sterile tweezers. Tissue was treated under laminar culture hood with gloved hands to eliminate contamination. Mechanical disaggregation was applied with a sterile scalpel then tumor digestive enzyme (ATP-Tumor Chemosensivity Assay; Innovative Diagnostic-Systeme, Germany) was added and incubated overnight 37ºC in a humidified incubator under 5% CO_2. After incubation, homogenized tissue was centrifuged (Universal 320 R; Hettich, Germany) 800 rpm in 8 min. Following to centrifuged supernatant was removed with a sterilized Pasteur pipette connected to a controlled, continuous vacuum pump system and the pellet resuspended with 1 mL CAM, then complete to 5 ml volume with CAM. Washing the pellet with this style was done twice to remove enzyme effect on pellet was eliminated by washing as explained above.

Then, 0.01 ml of cell suspension was added into 0.01 ml of tripan blue solution to measure the cell number by using the automatic cell counter (Invitrogen Cell Counting, IST, TR).

Primer human breast cancer cells ($1x10^4$) were seeded in microwell culture plate which must be the U shape to gain volume for growing (ATP-Tumor Chemosensivity Assay; Innovative Diagnostic-Systeme, Germany) and incubated 37ºC in a humidified incubator under 5% CO_2 in 2 days for cells to settle down.

Plates divided 10 groups (Figure 2.1). These are:

1. Control (C) : Primer Breast Cancer Cells Alone
2. ICG Alone : Primer Breast Cancer Cells and ICG (50 μM)
3. Light Alone: Primer Breast Cancer Cells and Light (780 nm, 35min, 18 mW/cm^2)
4. PDT : Primer Breast Cancer Cells, ICG (50 μM) and Light (780 nm, 35min, 18 mW/cm^2)
5. PDT+Paclitaxel : Primer Breast Cancer Cells, ICG (50 μM), Light (780 nm, 35min, 18 mW/cm^2) and 100 TDC Paclitaxel
6. PDT+Doxorubicin : Primer Breast Cancer Cells, ICG (50 μM), Light(780 nm, 35min, 18 mW/cm^2) and 100 TDC Doxorubicin
7. PDT+5Fluorouracil: Primer Breast Cancer Cells, ICG (50 μM), Light (780 nm, 35min, 18 mW/cm^2) and 100 TDC 5Fluorouracil
8. Paclitaxel : Primer Breast Cancer Cells and 100 TDC Paclitaxel
9. Doxorubicin : Primer Breast Cancer Cells and 100 TDC Doxorubicin
10. 5Fluorouracil : Primer Breast Cancer Cells and 100 TDC 5Fluorouracil

	1	2	3	4	5	6	7	8	9	10	11	12
A	ATP Inhibitory	ATP Inhibitory	ATP Inhibitory				CAM Alone	CAM Alone	CAM Alone	C	C	C
B												
C												
D	PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone			PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone
E	PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone			PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone
F	PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone			PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone
G	PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone			PDT+Paclitaxel	PDT+Doxorubicin	PDT+5Fluorouracil	PDT	Light Alone
H												

Figure 3.1 Well ID, Compound Name has shown for PDT and PDT combined chemotherapy application.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ATP Inhibitory	ATP Inhibitory	ATP Inhibitory				CAM Alone	Cam Alone	Cam Alone	C	C	C
B												
C												
D	Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone				Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone	
E	Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone				Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone	
F	Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone				Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone	
G	Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone				Paclitaxel	Doxorubicin	5Fluorouracil	ICG Alone	
H												

Figure 3.2 Well ID, Compound Name has shown for ICG Alone and Chemotherapy application.

3.2 Light Source

The light irradiation experiments were performed with 5x4 LED array system (Figure 3.2). LED system is connected to ARDUINO® open-source electronics prototyping platform that can be controlled by a computer. LEDs irradiate at 780 nm peak wavelength and have 18 mW optical powers measured by optical power and energy meter (S121C Standart Photodiode Power Sensor, Si, 400-1100 nm, 500 mW, PM200 Optical Power and Energy Meter Thorlabs). It did not show any temperature increase with continuous opening in 45 minutes on LED's.

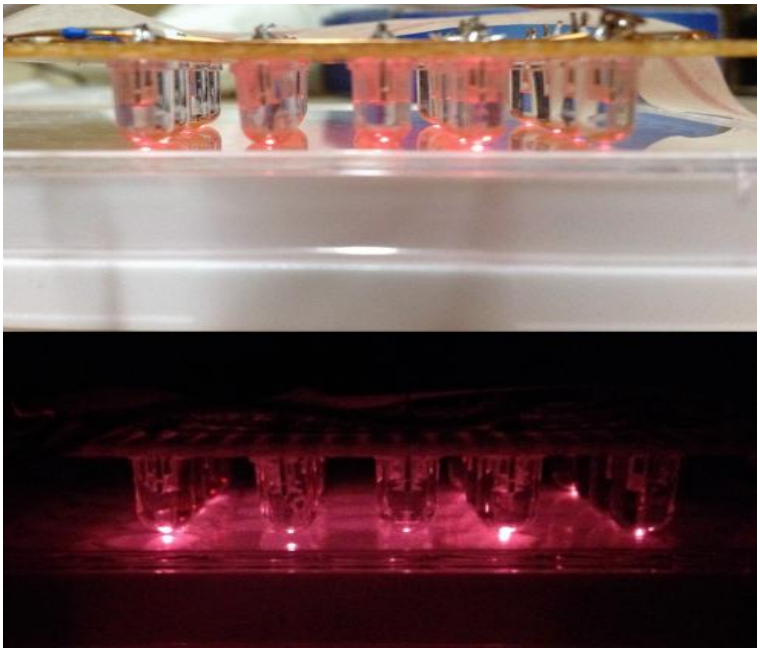

Figure 3.3 Picture show LED based illumination system

3.3 Photodynamic Therapy and Photodynamic Therapy Combined Chemotherapy Protocols

PDT and PDT combined chemotherapy protocols were applied following to incubated 37ºC in a humidified incubator under 5% CO_2 in 2 days. ICG alone group, PDT group and PDT-combined chemotherapy groups were incubated in 50 µM ICG (ICG-Pulsion, Germany) in 24 hour. Paclitaxel group was incubated with 100 TDC paclitaxel in 48 hour. Doxorubicin group was incubated with 100 TDC doxorubisicin in 48 hour. 5Fluorouracil group was incubated 100 TDC 5fluororucail in 48 hour. 100 After 24 hour, light alone group, PDT group and PDT-combined chemotherapy groups were treated under 780 nm LED for 35 minute with a power density 18 mW/cm^2. 18 mW optical powers measured by optical power and energy meter (S121C Standart Photodiode Power Sensor, Si, 400-1100 nm, 500 mW, PM200 Optical Power and Energy Meter Thorlabs). Right after that PDT-combined chemotherapy groups were to applied 100 TDC paclitaxel (Pharmaplan, South Africa), doxorubicin (MED İlaç, Turkey) and 5fluorouracil (Koçak Farma, Turkey). Plates were kept dark after ICG added during all therapy protocols.

3.4 Analysis Methods

Primer human breast cancer cells ($1x10^4$) were seeded in microwell culture plate and after treatment, application plate was incubated three days 37ºC in a humidified incubator under 5% CO_2. Following to incubation 50 µL Tumor Cell Extraction Reagent (Tumor Cell Extraction Reagent; ATP-Tumor Chemosensivity Assay; Innovative Diagnostic-Systeme, Germany) were added to plate, pipeting 5 times and 20 minutes incubated in room temperature, respectively.

All wells in microwell culture plate were transfered to white plate after 3 times pipeting, then 50 µL Luciferin-Luciferase (Luciferin-Luciferase; ATP-Tumor Chemosensivity Assay; Innovative Diagnostic-Systeme, Germany) was added to white plate. Immedialty cell viability was measured by using microplate luminometer (Microplate Luminometer; Centro LB 962 Microplate Luminometer, Germany) (Figure 3.4).

Figure 3.4 The microplate luminometer system

CHAPTER 4

RESULTS AND DISCUSSION

ICG mediated PDT and PDT combined chemotherapy effect on human primer breast cancer cells measured after treatment by using microplate luminometer. Graphs observed in the figure represent eight volunteer patient's surgery materials from independent experiments. All graphs have been obtained by using percent vitality.

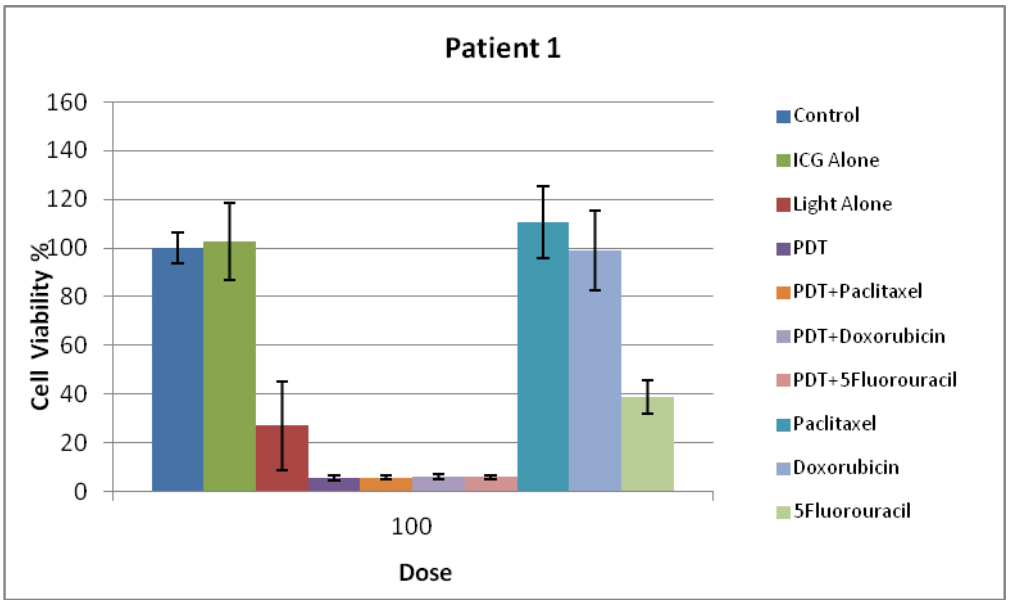

Figure 4.1 $1x10^4$ Primer breast cancer cells from Patient-1 seeded, incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 μM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC.

Patient 1's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.1);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50μM concentration. %102,69 ± cell viability has been shown with 50 μM incubation with 2 days.

For Light Alone Group; %26,18 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradition.

For PDT Group; 50μM concentration ICG application with PDT application 35 minutes induced %94,26 ± decreasing on viability.

For PDT+Paclitaxel Group; 100TDC paclitaxel application following to 50μM concentration ICG application with PDT application 35 minutes induced %94,25 ± decreasing on viability.

For PDT+Doxorubixin Group; 100TDC doxorubicin application following to 50μM concentration ICG application with PDT application 35 minutes induced %93,95 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %94,11 ± decreasing on viability.

For Paclitaxel Group; %110,47 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %99,12 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %38,83 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50μM concentration ICG didn't show any reducing on viability. For the light alone group results, after 35 min irradiation unexpectedly viability significant reduced. We can say that may be light source caused to partial photothermal effect on cells. According to chemotherapeutic drugs viability result, 5fluorouracil is the most effective drug for patient-1. 100 TDC paclitaxel increased the proliferations.

50µM ICG with 35 min light irradiation was significantly reduced to viability. Despite paclitaxel group, PDT-Paclitaxel combined application was very effective on cells. PDT-Doxorubisin and PDT-5Fluorouracil groups were effective too.

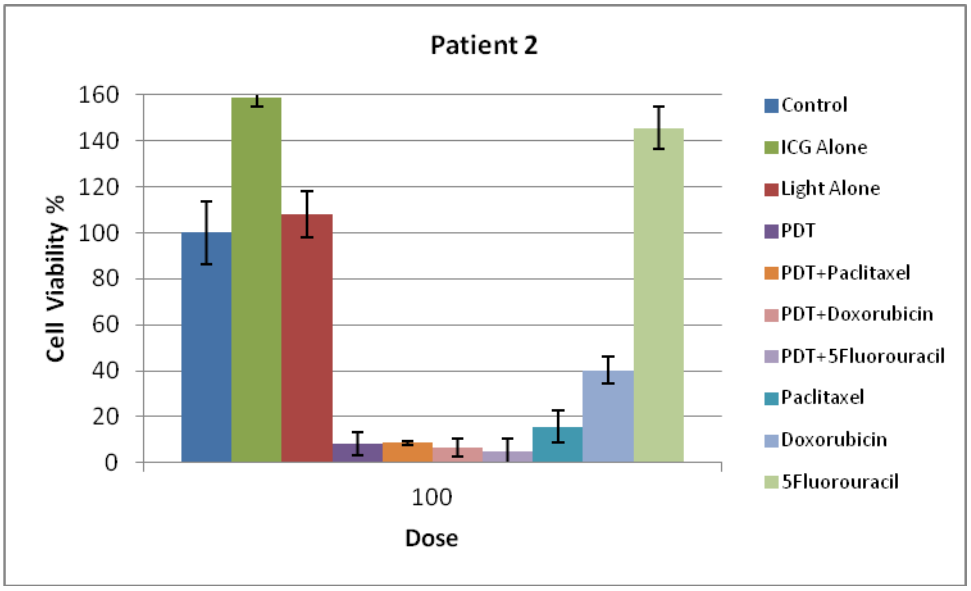

Figure 4.2 $1x10^4$ Primer breast cancer cells from Patient-2 seeded, incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 2's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.2);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50µM concentration. %159 ± cell viability has been shown with 50 µM incubation with 2 days.

For Light Alone Group; %108,4 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradition. Light wasn't shown any toxic effect.

For PDT Group; 50µM concentration ICG application with PDT application 35 minutes induced %92 ± decreasing on viability.

For PDT+Paclitaxel Group; 100TDC paclitaxel application following to 50µM concentration ICG application with PDT application 35 minutes induced %91 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50μM concentration ICG application with PDT application 35 minutes induced %93,8 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %95,1 ± decreasing on viability.

For Paclitaxel Group; %16 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %40 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %146 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50μM concentration ICG showed increasing effect on viability. It can be said that ICG have no toxic effect without light irradiation. For the light alone group results, viability wasn't decreased significantly. According to chemotherapeutic drugs viability result, paclitaxel was most effective drug for patient-2. 100 TDC 5Fluorouracil increased the proliferations.

50μM ICG with 35 min light irradiation %92 reduced the viability. PDT-5Fluorouracil combined application was most effective therapy group compared to 5Fluorouracil group. Viability was significantly reduced by PDT-Doxorubisin and PDT-Paclitaxel treatments.

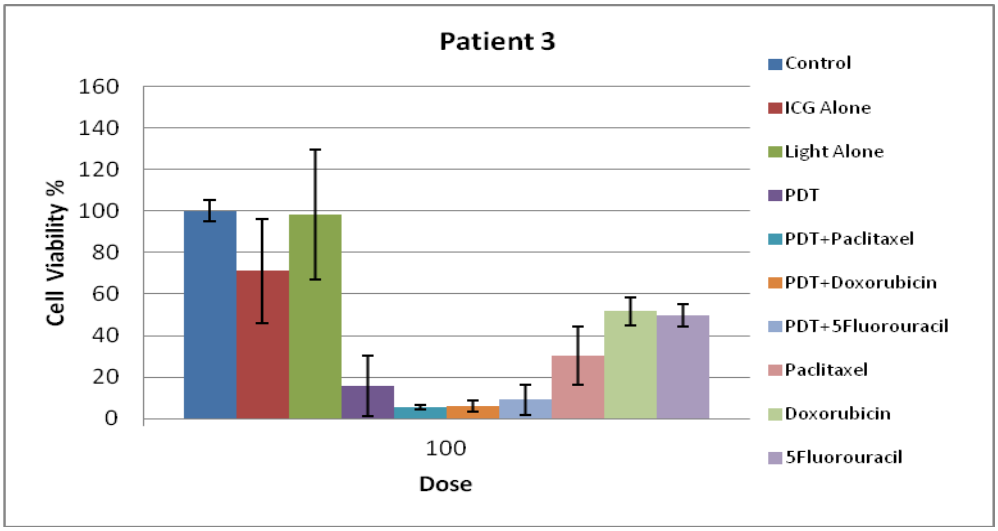

Figure 4.3 $1x10^4$ Primer breast cancer cells from Patient-3 seeded, incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 3's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.3);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50µM concentration. %71,06 ± cell viability has been shown with 50 µM incubation with 2 days.

For Light Alone Group; %98,163 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradiation. Light wasn't shown any toxic effect.

For PDT Group; As cell viability results ICG application with PDT application 35 2cminutes were have been effective on primer breast cancer cells. %15,68 ± cell viability has been shown.

For PDT+Paclitaxel Group; 100TDC paclitaxel application following to 50µM concentration ICG application with PDT application 35 minutes induced %94,5 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50µM concentration ICG application with PDT application 35 minutes induced %94 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50µM concentration ICG application with PDT application 35 minutes induced %91,1 ± decreasing on viability.

For Paclitaxel Group; %30,12 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %51,79 ± cell viability has been shown with 100 TDC doksorubicin applications.

For 5Fluorouracil Group; %49,89 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50µM concentration ICG had no significant effect on viability. For the light alone group, had no effect on viability. According to chemotherapeutic drugs viability result, paclitaxel showed %69,88 reduced effect for patient-3. 100 TDC Doxorubicin and 100 TDC 5Fluorouracil didn't showed important effect, cell viability measured respectiviliy %51,79 and %49,89.

50µM ICG with 35 min light irradiation was significantly reduced to viability. Viability of PDT-Paclitaxel combined application was significantly reduced %5,5. Also, viability was significantly reduced to %6 and %8,9 by PDT-Doxorubisin and PDT-5Fluorouracil treatments.

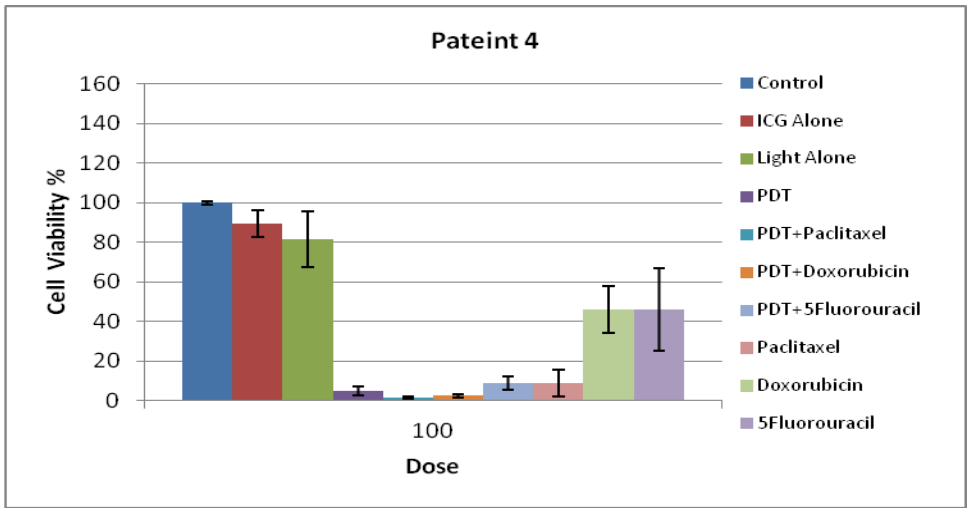

Figure 4.4 $1x10^4$ Primer breast cancer cells from Patient-4 seeded incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 4's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.4);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50μM concentration. %89,25 ± cell viability has been shown with 50 μM incubation with 2 days.

For Light Alone Group; %81,58 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradiation. Light wasn't shown any toxic effect.

For PDT Group; As cell viability results ICG application with PDT application 35 minutes were have been effective on primer breast cancer cells. %4,83 ± cell viability has been shown.

For PDT+Paclitaxel Group; As cell viability results ICG application with PDT application 35 minutes and 100TDC paclitaxel application were have been effective on primer breast cancer cells. PDT+Paclitaxel application induced %98,51 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50μM concentration ICG application with PDT application 35 minutes induced %97,63 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %91,1 ± decreasing on viability.

For Paclitaxel Group; %9 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %46 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %46 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50μM concentration ICG had no significant effect on viability. For the light alone group, irradiation has no effect on viability. According to chemotherapeutic drugs viability, paclitaxel was %9 highly effective for patient-4. 100 TDC Doxorubicin and 100 TDC 5Fluorouracil didn't show important reduced effect on primer breast cancer cells viability.

50µM ICG with 35 min light irradiation was significantly %4,83 reduced the viability. PDT-Paclitaxel combined application was most effective therapy group. PDT-Paclitaxel combined effect destroyed almost all breast cancer cells, viability measured %1,49. Viability was significantly reduced to %2,37 and %8,9 by PDT-Doxorubisin and PDT-5Fluorouracil treatments.

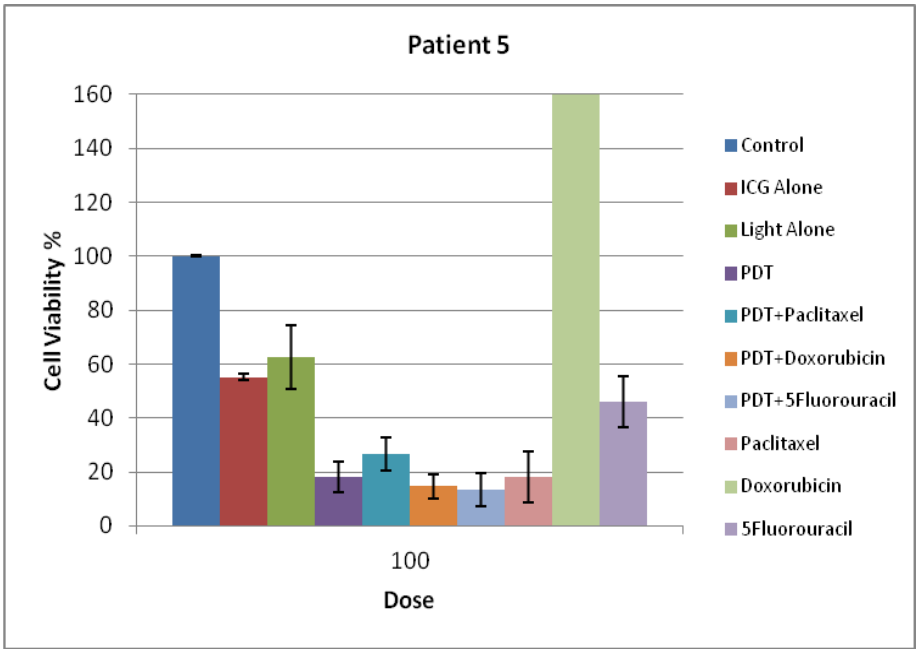

Figure 4.5 $1x10^4$ Primer breast cancer cells from Patient-5 seeded incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 5's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.5);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50µM concentration. %55,18 ± cell viability has been shown with 50 µM incubation with 2 days.

For Light Alone Group; %62,77 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradiation. Light wasn't shown any toxic effect.

For PDT Group; As cell viability results ICG application with PDT application 35 minutes were have been effective on primer breast cancer cells. %18,14 ± cell viability has been shown.

For PDT+Paclitaxel Group; PDT and 100 TDC Paclitaxel application induced %73,23 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50μM concentration ICG application with PDT application 35 minutes induced %85,38 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %86,78 ± decreasing on viability.

For Paclitaxel Group; %18 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %178 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %46 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50μM concentration ICG showed reduced effect on cell viability. For the light alone group results, viability wasn't show significant decreased significantly. According to chemotherapeutic drugs viability result, 100TDC paclitaxel showed significantly reduced to %18. 100TDC Doxorubicin wasn't showed any reduced effect. Contrary doxorubicin showed stimulative effect on primer breast cancer cells.

50μM ICG with 35 min light irradiation was %81,86 reduced the viability. PDT-5Fluorouracil combined application was most effective therapy group. PDT-Doxorubisin and PDT-Paclitaxel groups were significantly reduced to viability.

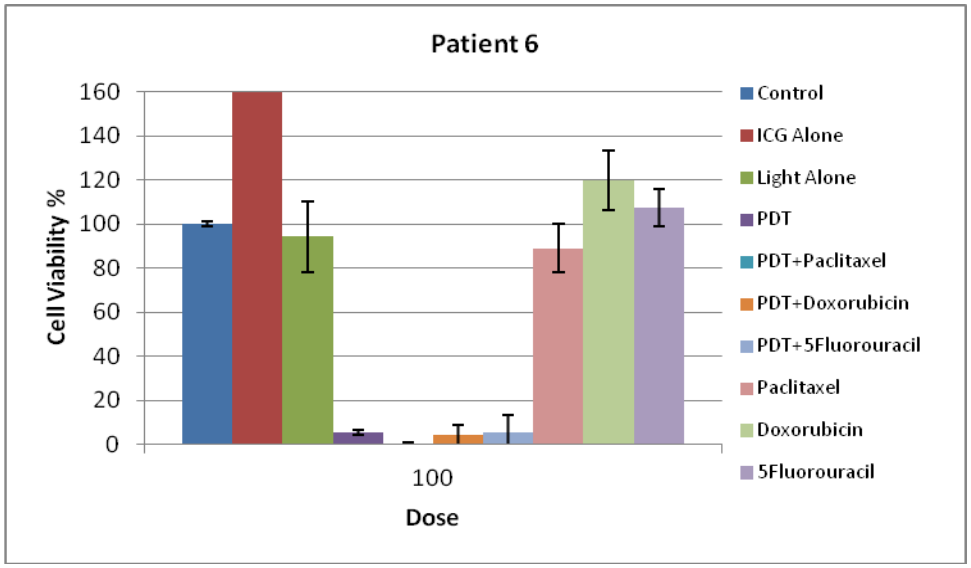

Figure 4.6 1x10^4 Primer breast cancer cells from Patient-6 seeded incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 6's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.6);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50µM concentration. %165 ± cell viability has been shown with 50 µM incubation with 2 days.

For Light Alone Group; %94,24 ± cell viability has been shown with 780 nm, 35minutes and 18 mw/cm^2 irradiation. Light wasn't shown any toxic effect.

For PDT Group; As cell viability results ICG application with PDT application 35 minutes were have been effective on primer breast cancer cells. %5,40 ± cell viability has been shown.

For PDT+Paclitaxel Group; PDT and 100 TDC Paclitaxel application induced %99,39 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50µM concentration ICG application with PDT application 35 minutes induced %93,97 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50µM concentration ICG application with PDT application 35 minutes induced %94,59 ± decreasing on viability.

For Paclitaxel Group; %89,14 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %120,03 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %107,27 ± cell viability has been shown with 100 TDC 5Fluorouracil applications.

50µM concentration ICG showed increasing effect on viability. It can be said that ICG have no toxic effect without light irradiation. For the light alone group results, irradiation wasn't show significant reduced on viability. According to chemotherapeutic drugs viability result, paclitaxel, doxorubicin and 5fluorouracil weren't effective for patient-6. 100TDC doxorubicin and 100TDC 5fluorouracil showed stimulative effect on viability.

50µM ICG with 35 min light irradiation %94,6 reduced the viability. PDT-paclitaxel combined application was most effective therapy group. PDT-Paclitaxel combined effect destroyed all breast cancer cells. Viability was significantly reduced by PDT-Doxorubisin and PDT-5Fluorouracil treatments.

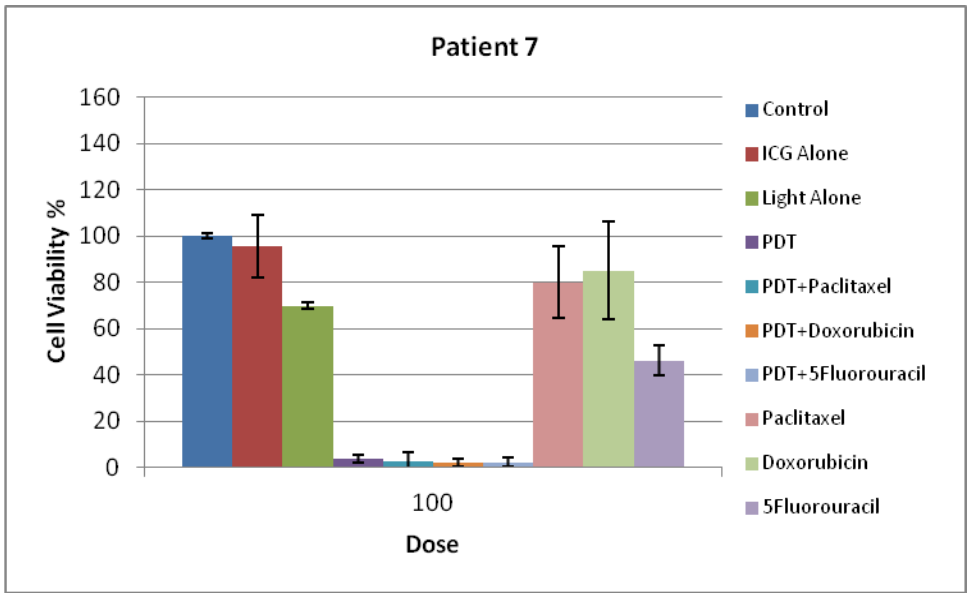

Figure 4.7 $1x10^4$ Primer breast cancer cells from Patient-7 seeded incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC

Patient 7's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.7);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50μM concentration. %95,73 ± cell viability has been shown with 50 μM incubation with 2 days.

For Light Alone Group; %69,83 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradiation. Light wasn't shown any toxic effect.

For PDT Group; As cell viability results ICG application with PDT application 35 minutes were have been effective on primer breast cancer cells. %3,64 ± cell viability has been shown.

For PDT+Paclitaxel Group; PDT and 100 TDC Paclitaxel application induced %97,24 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50μM concentration ICG application with PDT application 35 minutes induced %97,96 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %97,69 ± decreasing on viability.

For Paclitaxel Group; %80 ± cell viability has been shown with 100 TDC paclitaxel applications.

For Doxorubicin Group; %85,2 ± cell viability has been shown with 100 TDC doxorubicin applications.

For 5Fluorouracil Group; %46,3 ± cell viability has been shown with 100 TDC 5Fluorouracil application.

50μM concentration ICG wasn't show reduced effect on viability. It can be said that ICG have no toxic effect without light irradiation. Light alone group wasn't show significant reduced effect on viability. According to chemotherapeutic drugs viability result, 5fluorouracil reduced effect to %46,3. Viability wasn't significantly reduced by 100 TDC doxorubicin and 100TDC 5Fluorouracil.

50µM ICG with 35 min light irradiation %96,36 reduced the viability. PDT-paclitaxel, PDT-Doxorubisin and PDT-5fluorouracil combined groups' effect destroyed almost all breast cancer cells.

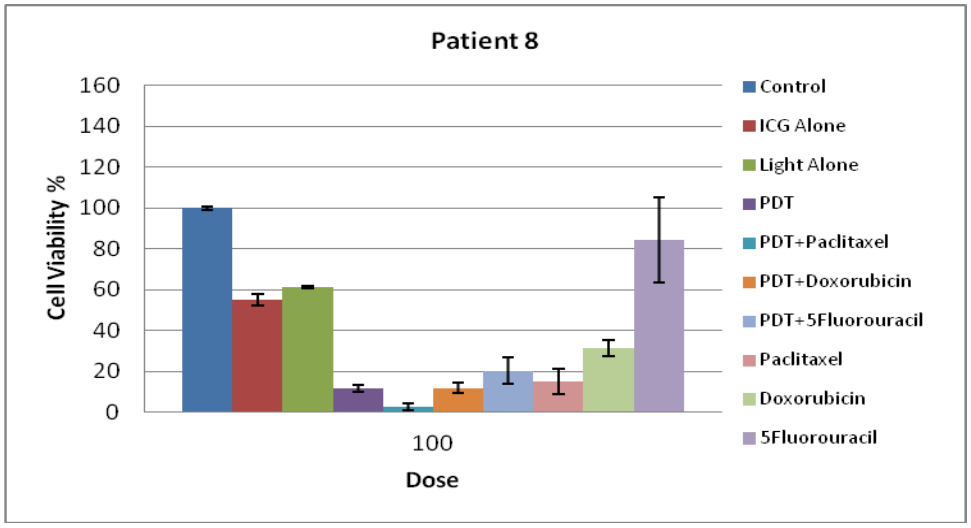

Figure 4.8 $1x10^4$ Primer breast cancer cells from Patient-8 seeded incubated and treated. Cell viability observed, (x) axis shows dose and (y) axis shows cell viability per cent. ICG was applied 50 µM and paclitaxel, doxorubicin and 5fluorouracil were applied 100 TDC.

Patient 8's PDT and PDT Combined Chemotherapy Treatment Results (Figure 4.8);

For ICG Alone Group; Vitality results weren't shown any toxic effect at 50µM concentration. %55,24 ± cell viability has been shown with 50 µM incubation with 2 days.

For Light Alone Group; %61,05 ± cell viability has been shown with 780 nm, 35minutes and 18 mW/cm^2 irradition.

For PDT Group; 50µM concentration ICG application with PDT application 35 minutes induced %88,5 ± decreasing on viability.

For PDT+Paclitaxel Group; 100TDC paclitaxel application following to 50µM concentration ICG application with PDT application 35 minutes induced %97,52 ± decreasing on viability.

For PDT+Doxorubicin Group; 100TDC doxorubicin application following to 50µM concentration ICG application with PDT application 35 minutes induced %88,29 ± decreasing on viability.

For PDT+5Fluorouracil Group; 100TDC 5Fluorouracil application following to 50μM concentration ICG application with PDT application 35 minutes induced %79,72 ± decreasing on viability.

For Paclitaxel Group; %14,97 ± cell viability has been shown with 100 TDC paclitaxel application.

For Doxorubicin Group; %31,37 ± cell viability has been shown with 100 TDC doxorubicin application.

For 5Fluorouracil Group; %84,44 ± cell viability has been shown with 100 TDC 5Fluorouracil application.

50μM concentration ICG showed reduced effect on viability. For the light alone group results, viability wasn't decreased significantly. According to chemotherapeutic drugs viability result, paclitaxel was significantly effective for patient-5. 100TDC doxorubicin was reduced effect on viability. 100TDC 5fluorouracil wasn't showed important reduced effect.

50μM ICG with 35 min light irradiation %88,5 reduced the viability. PDT-Paclitaxel combined application was most effective therapy group. Viability was significantly reduced by PDT-Doxorubisin and PDT-Paclitaxel treatments.

Patient Number	Pathology	Sub Type	Grade	Bl-Rc	Diameter	Vascular-invasion	Lymphatic-invasion	Nodular-invasion	Estrogen	Progestrone	Erb b2	Ki67	T	N
Patient 1	Ductal	Lum A	2	7	35	0	0	0	90	40	1	20	2	0
Patient 2	Ductal	Her 2							50	0	3	30		
Patient 3	Ductal	Lum A	3	8	45	1	1	0	90	50	1	20	4b	1
Patient 4	Lobüler	Lum A	2	6	40	1	1	0	95	25	0	20	2	1
Patient 5	Mucinous	Lum A	1	5	35	0	0	0	90	0	0	15	2	0
Patient 6	Duktal	Lum B	3	8	45	1	1	1	95	30	0	30	4	1
Patient 7	Duktal	Lum B	3	9	16	1	1	0	90	20	2	40	1c	2a
Patient 8	Ductal	Triple Neg	3	9	55	1	1	0	0	0	2	75	3	1

Figure 4.9 Patients pathology results

According to paired samples test results, there is no significant differences between PDT and PDT-Combined therapy groups (Figure 4.10).). Significance level for the PDT between PDT-Paclitaxel comparison p values 0,311; PDT between PDT-Doxorubicin comparison p values 0,462; PDT between PDT-5Fluorouracil comparison p values 0,852.

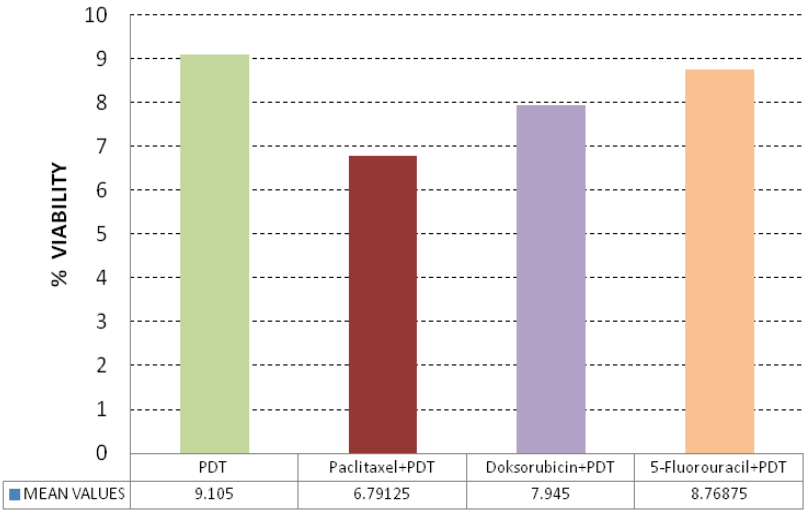

Figure 4.10 Viability of primer breast cancer cells treated with ICG mediated PDT and ICG mediated PDT combined Paclitaxel, ICG mediated PDT combined Doxorubicin and ICG mediated PDT combined 5Fluorouracil

According to paired samples test result, there are significant difference was foundes between PDT and Chemotherapy therapy groups (Figure 4.11).). Significance level for the PDT between Paclitaxel group comparison p values 0,048; PDT between Doxorubicin group comparison p values 0,004; PDT between 5Fluororuracil group comparison p values 0,003.

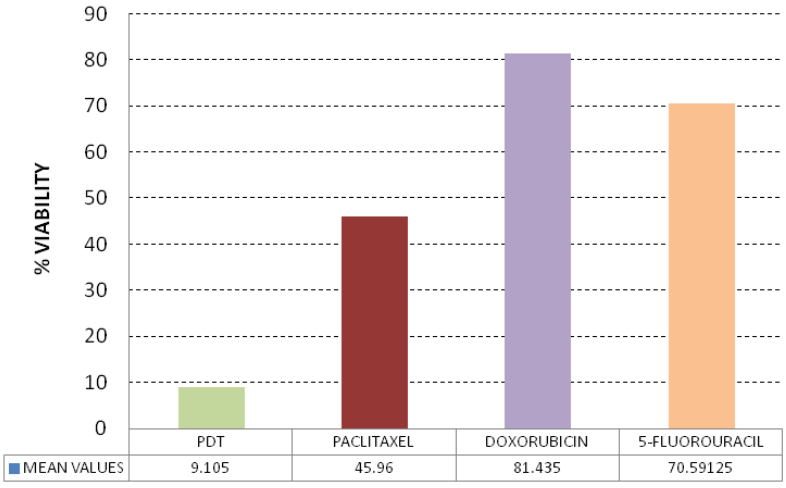

Figure 4.10 Viability of primer breast cancer cells treated with ICG mediated PDT and Paclitaxel, Doxorubicin and 5Fluorouracil.

CONCLUSIONS AND RECOMMENDATIONS

PDT is a minimally invasive and approved clinical cancer therapy method. PDT has minimal side effects other than traditional cancer treatment methods, such as surgery, radiotherapy and chemotherapy and most importantly; it is repetable. PDT success depends on the photosensitizers' type and optimal concentration, light dose and rate of irradiation at the target point and oxygen concentration at that specific point [54]. ICG mediated PDT has been reported to be effective on various cancer cells [67, 94-96]. This thesis study investigated response of human primer breast cancer cells to PDT, chemotherapy and PDT combined chemotherapy. The result of the study showed that ICG mediated PDT and ICG mediated PDT combined chemotherapy reduced viability of human breast cancer cells ($p=0.002$).

Microplate luminometer system measurement was used as golden standard in order to confirm that ICG, paclitaxel, doxorubicin and 5fluorouracil reduce cell viability. When human primer breast cancer cells were treated with ICG alone then incubated for 24 hour, ICG didn't show any toxic effect on the cells. The ICG concentration of 50 μM was determined to non-cytotoxic dose. 35 minutes, 18 mW LED irradiation didn't show cytotoxic effect when primer human breast cancer cells incubated after LED irradiation for 24 h. Human primer breast cancer cells were treated with a 50μM ICG incubated 24 h, after than irradiated with 18 mW 780 nm LED system cells viability reduced significantly cell viability ($p=0.002$).

Bozkulak and *et al* were studied ICG mediated PDT on MDA-MB231 human breast cancer cells. MDA-MB321 cells irradiated with 809 nm 60 mW diode laser priorly incubated with 50μM ICG at 24 h. They showed 50μM ICG was effective and non cyctotoxic PDT application dose [94]. Abo Zeid and *et al* were showed that when MCF-7 human breast adenocarcinoma cells were incubated with 200μM ICG at 48 h and irradiated with 807 nm diode laser 400 mW 20 min, cell viability significantly reduced ($p<0.01$) [68]. Tseng and *et al* were studied ICG mediated PDT on MIA PaCa-2, PANC-1, and BxPC-3 human pancreatic cancer cells. In all 3 cancer cell lines, significant growth inhibition was observed at 10 μg/mL ICG with nearly total ablation at 20 μg/mL ICG ($p< 0.01$) [70].

In the literature there are some studies about optimal ICG concentration and energy of appliying light for standart cell lines. However, as our knowledge, there are no optimal parameters for primer human cancer cells. This study will be preliminary step for the future clinical applications.

Chemotherapy alone groups didn't show significant cytotoxic effect on human primer breast cancer cells (for all chemotherapy alone groups $p>0.05$). On the other hand, PDT combined chemotherapy treatment showed a difference between the three chemotherapy alone groups. All the combine groups significantly reduced to cell viability (for all PDT combined chemotherapy groups ($p<0.0001$). PDT combined paclitaxel treatments were significantly reduced to viability %6,791 and it's founded most effective therapy groups. PDT and chemotherapy showed synergic interaction.

For the clinical applications PDT combined chemotherapy has many of advantages. These are minimized side effect, higher success rate and less cost effect. Combined therapy optimal photosensitizer concentration and chemical drugs concentrations will be chose depend on patient's age, tumor type and pathlogy.

In the future advanced clinic applications PDT can be combined with surgery. Firstly if patient is operable, tumor bed will be cleaned and PDT can appled like a protective treatment [97].

Next step of this study determination of the shape of the cell death and investigation of TP53, HER-2 and TOP2A genes signals by using interphase fluorescence in situ hybridization (nuc-FISH).

Further *in vivo* experimental studies have beeen planed to evaluate ICG mediated PDT to observe show potential therapeutic approach effect for breast cancer treatments.

REFERENCES

[1]Verdecchia A, G.S., Francisci S, et al., *Survival trends in European cancer patients diagnosed from 1988 to 1999.* European Journal of Cancer, 2009. 45(6): p. 1042-66.

[2]Rebecca Siegel, D.N., Ahmedin Jemal, *Cancer Statistics, 2013.* CA CANCER J CLIN 2013. 63: p. 11–30.

[3]C.A. Morton, K.E.M., L.E. Rhodes, *Guidelines for topical photodynamic therapy: update.* British Journal of Dermatology, 2008. 159 p. 1245–1266.

[4]R.R. Allison, H.C.M., C.H. Sibata, *Clinical PD/PDT in North America: An historical review.* Photodiagnosis and Photodynamic Therapy, 2004. 1: p. 263,377.

[5]Gomer, C.J., *Photodynamic Therapy: Methods and Protocols*, ed. J. M.Walker. 2010: Springer.

[6]Clemens Fritsch, G.G., Thomas Ruzicka, *Photodynamic Therapy in Dermatology.* American Medical Association, 1998 134: p. 207-214.

[7]Bissell MJ, Radisky D. 2001. Putting tumors in context. *Nat. Rev. Cancer* 1:46–54.

[8]Tlsty TD., 2001, *Stromal cells can contribute oncogenic signals*, Semin. Cancer *Biol.*11:97–104.

[9]Mueller MM, Fusenig NE., 2004, *Friends or foes bipolar effects of the tumour stroma in cancer*. *Nat. Rev. Cancer* 4:839–49.

[10]Van den Hooff A., 1988, *Stromal involvement in malignant growth,* Adv. Cancer Res, 50:159–96.

[11]McCormick F., 2004, *Cancer: survival pathways meet their end,* Nature 428:267–69.

[12]Fitzpatrick FA. 2001. *Inflammation, carcinogenesis and cancer.* Int. Immuno. 1:1651–67.

[13]Coussens LM,Werb Z. 2002. *Inflammation and cancer*. *Nature* 420:860–67.

[14]Macdonald, F.C. Ford, and A. Casson, *Molecular biology of cancer*, New York: BIOS Scientific, 2nd ed. 2004.

[15]Ronnov-Jessen L, Petersen OW, Bissell MJ. 1996. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. *Physiol. Rev.* 76:69–125.

[16] Mackie EJ, Chiquet-Ehrismann R, Pearson CA, Inaguma Y,Taya K, et al. 1987.Tenascinis a stromal marker for epithelial malignancy in the mammary gland. *Proc. Natl. Acad. Sci. USA* 84:4621–25.

[17] Rasmussen AA, Cullen KJ. 1998. Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors. *Breast Cancer Res. Treat.* 47:219–33.

[18]Pecorino, L., Molecular biology of cancer: mechanisms, targets, and therapeutics, Oxford: Oxford University Press, 2005.

[19]Institute, N. C., Chemotherapy and you. A guide to self-help during cancer treatment, USA: National Institutes of Health, 2007.

[20]Institute, N. C., *Radiation Therapy and you. A guide to self-help during cancer treatment*, USA: National Institutes of Health, 2007.

[21]Panno, J., *Cancer: The role of genes, lifestyle, and environment*, New York: NY Facts On File Inc., 2005.

[22]Dennis E. J., G. J. Dolmans, D. Fukumura, and R. K. Jain, *Photodynamic therapy for cancer*, Nat Rev Cancer, Vol. 3, pp. 380-387, 2003.

[23]Kessel D., *Photodynamic therapy: from the beginning*, Photodiagnosis Photodynamic Ther., Vol. 1, pp. 3-5, 2004.

[24]Hopper C., *Photodynamic therapy: a clinical reality in the treatment of cancer*, Lancet Oncol., Vol. 1: 212–19, 2000.

[25]Ackroyd R., Kelty C., Brown N., Reed M., *The history of photodetection and photodynamic therapy*, Photochem-Photobiol, 2001;74(5):656-69.

[26]Finsen NF., *Phototherapy*, London, Arnold; 1901.

[27]Fitzpatrick TB., Pathak MA., *Historical aspects of methoxsalen and other furocoumarins*, J Invest Dermatol 1959;32(2, Part 2):229-31.

[28]Hausmann W., *Die sensibilisierende Wirkung des haematoporphyrins*, Biochem Z 1911;(30):276-316.

[29]Raab O., *Uber die Wirkung fluoreszierender Stoffe auf Infusorien*, Z Biol, 1900;39:524-46.

[30] Prime J*., Les accidentes toxiques par l'eosinate de sodium*, Paris: Jouve and Boyer, 1900.

[31]Von Trappeiner H., Jesionek A., *Therapeutische Versuche mit fluoreszierenden Stoffen.*, Med Worchenschr 1903;(47):2042-4.

[32]Meyer W., VI. The surgery of the pulmonary artery, Ann Surg, 1913;58(2):188-205.

[33]Kufe D. W., R. E. Pollock, R. R. Weichselbaum, R. C. Bast, and T. S. Gansler, Cancer Medicine, USA: BC Decker Inc., 6th ed., 2003.

[34]Moan J., Johannessen JV., Christensen T., Espevik T., McGhie JB., *Porphyrin-sensitized photoinactivation of human cells in vitro*, Am J Pathol 1982;109(2):184-92.

[35]Peng Q., Moan J., Farrants G., Danielsen HE., Rimington C., *Localization of potent photosensitizers in human tumor LOX by means of laser scanning microscopy*, Cancer Lett, 1990;53(2-3):129-39.

[36]Peng Q., Moan J., *Correlation of distribution of sulphonated aluminium phthalocyanines with their photodynamic effect in tumour and skin of mice bearing CaD2 mammary carcinoma*. Br J Cancer 1995. 72(3):565-74.

[37]Ackroyd R., Kelty C., Brown N., Reed M.. *The history of photodetection and photodynamic therapy.* Photochem Photobiol, Vol. 74, no. 5, pp. 656-669, 2001.

[38]Von Trappeiner H., Jodlbauer A., *Uber Wirkung der photodynamischen (fluorieszierenden) Stoffe auf Protozan und Enzyme,* Dtsche Arch Klin Med, 1904;80:427-87.

[39]Von Trappeiner H., Jodlbauer A., *Die Sensibilisierende Wirkung fluorieszierender Substanzer, gesammte Unter-suchungen uber die photodynamische erscheinung*; 1907.

[40]Moan J., Streckyte G., Bagdonas S., Bech O., Berg K., *Photobleaching of protoporphyrin IX in cells incubated with 5-aminolevulinic acid*. Int J Cancer 1997. 70(1):90-7.

[41]Sibata CH, Colussi VC, Oleinick NL, Kinsella TJ., *Photodynamic therapy: a new concept in medical treatment*, Braz J Med Biol Res, 2000;33(8):869-80.

[42]O'Connor AE., Gallagher WM., Byrne AT., *Porphyrin and nonporphyrin photosensitizers in oncology: preclinical and clinical advances in photodynamic therapy*, Photochem Photobiol, 2009;85(5):1053-74.

[43]Gorman A., Killoran J., O'Shea C., Kenna T., Gallagher WM., O'Shea DF., *In vitro demonstration of the heavyatom effect for photodynamic therapy*, J Am Chem Soc 2004;126(34):10619-31.

[44]Allison RR., Mang TS., Wilson BD., *Photodynamic therapy for the treatment of nonmelanomatous cutaneous malignancies,* Semin Cutan Med Surg 1998;17(2):153-63.

[45]Castano A.P., Demidova T.N., Hamblin M.R., *Mechanisms in photodynamic therapy: Part two – photosensitizers, photochemistry and cellular localization*, Photodiag. Photodyn. Ther., 2004, 1;279–293.

[46]Juzeniene A., Moan J., *The history of PDT in Norway. Part one – identification of basic mechanisms of general PDT*, Photodiag. Photodyn. Ther., 2007, 4; 3–11.

[47]Ochsner M., *Light scattering of human skin: a comparison between zinic(II)-pthalocyanine and photofrin II*, J. Photochem. Photobiol.: B. Biol., 1996, 32; 3–9.

[48]Plaetzer K., Krammer B., Berlanda J., Berr F., Kiesslich T., *Photophysics and photochemistry of photodynamic therapy: fundamental aspects*, Laser. Med. Sci., 2008, 1; 1–15.

[49]Pazos MDC., Nader HB., *Effect of photodynamic therapy on the extracellular matrix and associated components,* Braz. J. Med. Biol. Res., 2007, 40; 1025-1035.

[50]Robertson CA., Hawkins ED., *Abrahamse H., Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT*, Journal of Photochemistry and Photobiology B: Biology, 2009, 96; 1–8.

[51]Castano AP., Demidova TN., Hamblin MR., *Mechanisms in photodynamic therapy: part three – photosensitizer pharmacokinetics, biodistribution, tumor localization and modes of tumor destruction*, Photodiag. Photodyn. Ther., 2005, 2; 91 106.

[52]Foote CS., *Definition of type I and type II photosensitized oxidation*, Photochem Photobiol 1991, 54; 659.

[53]Henderson BW., Gollnick SO., Snyder JW and et al., *Choice of oxygen-conserving treatment regimen determines the inflammatory response and outcome of photodynamic therapy of tumors,* Cancer Res 2004, 64; 2120–2126.

[54]Patrizia A., Kristian B., Keith AC. And et al., *Photodynamic Therapy of Cancer: An Update*, CA Cancer J Clin, 2011, 61; 250-281.

[55]Prasad P., *Introduction to Biophotonic*, Canada: John Wıley and Sons, Inc., 2003.

[56]Allison R.R., Downie G.H., Cuenca R., Hu X., and et al, *Photosentisizers in clinical PDT,* Photodiagnosis Photodyn Ther, Vol. 1, pp. 27-42, 2004.

[57]Bonnett R., Berenbaum M., *Porphyrins as photosensitizers*, Ciba Found Symp., 146: 40-53, 1989.

[58]Wagnieres GA., Star WM., Wilson BC., *In vivo fluorescence spectroscopy and imaging for oncological applications,* Photochem Photobiol., 68(5):603-32, 1998.

[59]Bonnett R., Berenbaum MC., *HpD–a study of its components and their properties,*Adv Exp Med Biol., 160:241-50, 1983.

[60]Mironov AF., Nizhnik AN., Nockel A Y., *Haematoporphyrin derivatives: an oligometric composition study*. J Photochem Photobiol B Biol, 4:297-306, 1990.

[61]Kessel D., Thompson P., Musselman B. and et al., *Probing the structure and stability of the tumor-localizing derivative of hematoporphyrin by reductive cleavage with LiAlH4*, Cancer Res., 47(17):4642-5, 1987.

[62]Fickweiler S, Szeimies RM, Abels C. And and et al., *Photosensitization of skin derived cell lines by Dimegin (2,4-di-(α-methoxyethyl)deuteroporphyrin IX) in vitro.*, Photodermatol Photoimmunol Photomed., 14: 125–131, 1998.

[63]Desmettre T., Devoisselle V., Soulie-Begu V. and et al. *Fluorescence properties and metabolic features of indocyanine gren*, Journal Francais d Ophtalmologie, 22: 1003-16, 1999.

[64]Polom K., Murawa D., Rho YS., Nowaczyk P., Hunerbein M., Murawa P., *Current trends and emerging future of indocyanine green usage in surgery and oncology: a literature review,* Cancer, 117:4812-22, 2011.

[65]Alacam B., Yazici B., Intes X., Nioka S., Chance B., *Pharmacokinetic-rate images of indocyanine green for breast tumors using near-infrared optical methods*, Physics in Medicine and Biology., 53:837-59, 2008.

[66]Desmettre T., Devoisselle J.M., and Mordon S. , *Fluorescence Properties and Metabolic Features of Indocyanine Green (ICG) as Related to Angiography*, Survey Of Ophthalmology ,Vol 45:1, 2000

[67]Bozkulak Ö., *Photofrin mediated and ICG mediated PDT in cancer treatment*, Doctor of Philisopy, Bogazici University, 2010.

[68]Mona A.M., Abo-Zeid, Liehrb T. And et al., *Molecular cytogenetic evaluation of the efficacy of photodynamic therapy by indocyanine green in breast adenocarcinoma MCF-7 cells*, Photodiagnosis and Photodynamic Therapy PDPDT-444; 9, 2012.

[69]Mamoona A.M., Gamal A.M. and et al., *In vitro efficiency and mechanistic role of indocyanine green as photodynamic therapy agent for human melanoma*, Photodiagnosis and Photodynamic Therapy, 6:105-116, 2009.

[70]Tseng WW., Saxton RE., Deganutti A., Liu CD., *Infrared Laser Activation of Indocyanine Green Inhibits Growth in Human Pancreatic Cancer*, Pancreas,Vol 27:3, 2003.

[71]El-Daly S.M., Gamal-Eldeen A.M., *Photodynamic therapeutic activity of indocyanine green entrapped in polymeric nanoparticles*, Photodiagnosis and Photodynamic Therapy, PDPDT-435:13, 2012.

[72]Panjehpour M., Overholt BF., Haydek JM., *Light sources and delivery devices for photodynamic therapy in the gastrointestinal tract*, Gastrointest Endosc Clin North America, 10:513-32, 2000.

[73]Mang TS., *Lasers and light sources for PDT: past, present and future*, Photodiagnosis and Photodynamic Therapy, 1:43-48, 2004.

[74]Alexiades-Armenakas M., *Laser-mediated photodynamic therapy*, Clin. Dermatol., 24:16–25, 2006.

[75]Juzeniene A., Nielsen KP., Moan J., Biophysical *aspects of photodynamic therapy*, Journal of Environ Pathol Toxicol Oncol., ;25:7-28, 2006.

[76]Molar extinction coefficients of oxy and deoxyhemoglobin compiled by Scott Prahl. URL: http://omlc.ogi.edu/spectra/hemoglobin

[77]Nioka S., Wen S., Zhang J. and et al, *Simulation study of breast tissue hemodynamics during pressure perturbation*, Oxygen Transport to Tissue XXVI 566:17-22, 2006.

[78]Whitehurst C., Byrne K., Moore JM., *Development of an alternative light source to lasers for photodynamic therapy: 1. Comparative in vitro dose response characteristics.* Lasers in Med Sci., 8:259—67. 1993.

[79]Wilson BC., *Photodynamic therapy: light delivery and dosage for second generation photosensitizers. In: Photosensitizing compounds: their chemistry, biology and clinical use*, Chichester: John Wiley & Sons, p. 60-73, 1989

[80]Chen D., Zheng H., Huang Z., Lin H. and et al, *Light-Emitting DiodeBased Illumination System for In Vitro Photodynamic Therapy*, International Journal of Photoenergy, P: 6, 2012.

[81]Hopper C., Niziol C., Sidhu M., *The cost effectiveness of foscan mediated photodynamic therapy (foscan-pdt) compared with extensive palliative surgery and palliative chemotherapy for patients with advanced head and neck cancer in the UK*, Oral Oncol, Vol. 40, pp. 372-382, 2004.

[82]Kubler A., Niziol C., Sidhu M., Dünne A., Werner J.A., *Analysis of cost effectiveness of photodynamic therapy with foscan (foscan-pdt) in comparison with palliative chemotherapy in patients with advanced head-neck tumors in Germany*, Laryngorhinootologie, Vol. 84, no. 10, pp. 725-732, 2005.

[83]Hur C., Nishioka N. S., Gazelle G. S., *Cost-effectiveness of photodynamic therapy for treatment of barrett's esophagus with high grade dysplasia*, Dig Dis Sci, Vol. 48, pp. 1273-1283, 2003.

[84]Goodman J., Walsh V., *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug*, Cambridge University Press, 2001.

[85]Rowinsky EK. Adn et al, *Phase II study of taxol in advanced epithelial malignancies*, Proceedings of the Association of Clinical Oncology, 7: 136, 1988.

[86]Ottaggio L., Bestoso F., Armirotti A., Balbi A., Damonte G., Mazzei M., Sancandi M., Miele M., *Taxanes from Shells and Leaves ofCorylus avellana*, Journal of Natural Products, 71(1): 58–60, 2008.

[87]Saville, M.W., Lietzau, J., Pluda, J.M., Wilson, W.H., Humphrey, R.W., Feigel, E. and et al., *Treatment of HIV-associated Kaposi's sarcoma with paclitaxel*, The Lancet, 346(8966): 26–8, 1995.

[88]Engels B., Dahm P., Jennewein S,, *Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production*, Metabolic Engineering, 10 (3-4): 201–6, 2008

[89]Krysko DV., Kaczmarek A., Krysko O., Heyndrickx L., Woznicki J, Bogaert P, Cauwels A., Takahashi N., Magez S., Bachert C., Vandenabeele P.J., *TLR-2 and TLR-9 are sensors of apoptosis in a mouse model of doxorubicin-induced acute inflammation*, Cell Death Differ. 18 (8): 1316–25, 2011.

[90]Longley DB., Harkin DP., Johnston PG., *5-Fluorouracil: mechanism of action and clinical strategies*, Nat. Rev. Cancer 3 (5): 330–8, 2003.

[91]Kato H., *Photodynamic therapy for lung cancer-a review of 19 years experience*, Journel of Photochem Photobiol B, Vol. 42, pp. 96-99, 1998.

[92]McCaughan J.S., Williams T.E., Bethel B.H., *Photodynamic therapy of endobronchial tumors*, Lasers Surgery Med, Vol. 6, pp. 336-345, 1986.

[93]Mennel S.N., Hausmann C., Meyer H., Peter S., Photodynamic *therapy for exudative hamartoma in tuberous sclerosis*, Arch Ophthalmol, Vol. 124, no. 4, pp. 597-599, 2006.

[94]Bozkulak O., Fahrioglu Yamacı R., Tabakoglu O.H., Gülsoy M., *Photo-toxic effects of 809-nm diode laser and indocyanine green on MDA-MB231 breast cancer cells*, Photodiagnosis and Photodynamic Therapy, 6:117-121, 2009.

[95]Mamoon A.-M., Gamal-Eldeen A.M., Ruppel M.E., Smith R.J., Tsang T., Miller L.M., *In vitro efficiency and mechanistic role of indocyanine green as photodynamic therapy agent for human melanoma*, Photodiagnosis and Photodynamic Therapy, 6:105-116, 2009.

[96]Abels C., Fickweiler S., Weiderer P., Bäumler W., Hofstädter F., Landthaler M., Szeimies R.-M., *Indocyanine green (ICG) and laser irradiation induce photooxidation*, Arch Dermatol Res, 292:404–411, 2000.

[97]Douplik A., *Combined Surgery and Photodynamic Therapy of Cancer*, Physics Procedia, 5:641–645, 2010.

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List of Publications and Patents:

▪ **Kiris A.**, and Uzun A., 2011 : Çark Deresi'nde Toplam Koliform Bakteri ve *E.coli* Tayini. Undergraduate Thesis. June, 2011 Sakarya, TURKEY.

- **Kiris A.**, Kiris T., Akbulut S., Hasbahceci M., Gucin Z., Tabakoglu O. H., Muslumanoglu M, 2013: ICG kemoterapötik bir ajan mıdır. Tıp Teknolojileri Ulusal Kongresi – Biyomedikal ve Klinik Mühendisliği Derneği, November, 2013 Antalya, TURKEY.

- Bireller S., Rencizogulları C., **Kiris A.**, Cakmakoglu B., 2013: Investigation On Corilagin And Photodynamic Therapy On Braın Glioblastoma Cell Lines, November, 2013, TURKEY.

- **Kiris A.**, Kiris T., Akbulut S., Malya U., Gucin Z., Karatepe O., Tabakoglu O. H., 2013: Çift Toplayıcı Küre Sistemi İle Tümörlü Ve Sağlıklı Pankreas Dokusunun Optik Özelliklerinin Karakterizasyonu: Prospektif Klinik Çalışmanın Erken Dönem Sonuçları, November, 2013 Antalya, TURKEY.