Optical characterizations of synthetic nano PcGaCl thin film. In vitro, hemocompatibility, cytotoxicity, and PDT evaluation

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Doped Phthalocyanines compounds are very applicable materials that have been utilized in different fields of industry and medicine such as photovoltaic, fuel cell, and photodynamic therapy. Here, we report the optical, cytotoxicity, and PDT evaluation of PcGaCl thin film. The optical studies show that the investigated thin film has a high light abortion at a wavelength of 500 nm. the energy gap of PcGaCl thin film has been evaluated using the WDD model and it was found to be 3.9 eV. The hemocompatibility and cytotoxicity studies of PcGaCl thin film have been evaluated in Caco-2, melanoma, and fibroblast. PcGaCl thin film showed no cytotoxicity in the dark. Upon exposure to different radiation doses of 2.5 J/cm2, 4.5 J/cm2, and 8.5 J/cm2, PcGaCl thin film has exhibited a high photocytotoxicity. The result suggests that Ga(III)Cl thin film can be considered a promising photosensitizer for Photodynamic therapy (PDT).

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Keywords: Phthalocyanines, Thin-film, Optical properties, Hemocompatibility, Cytotoxicity, Photodynamic therapy

1. Introduction

Phthalocyanines (Pcs) are highly conjugated macrocycles that can form complexes with many different metals. Pcs have high chemical durability and high light resistance and are widely used in many electro-optical applications such as data storage, and chemical sensor production and are also used in photodynamic therapy (PDT)[1-4].

Pcs and their metal complexes are used as photosensitizers in PDT which provides an option for cancer treatment and other diseases[5, 6]. PDT treatment depends on the generation of reactive oxygen species (ROS) such as singlet oxygen and free radicals and they are responsible for killing the cancer cells. Water-soluble Pcs and their metal complexes have the ability for generating ROS upon light activation; in the near-IR region (670 - 750 nm) they can penetrate deeply into living tissues[7-9]. Another important propriety of Pcs is that they can accumulate in the tumorous tissue, and then are activated by light exposure to destroy cancer cells[8-10].

Gallium (III)-Phthalocyanine Chloride (PcGaCl) is a fluorescent organic infrared dye that can use in different applications, for example, optical applications and biomedical imaging[11, 12]. PcGaCl belongs to the cyanine family and has a chemical formula of $C_{32}H_{16}ClGaN_8$. Members of this family have been shown to interact with DNA and other relevant

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biomolecules[13, 14]. PcGaCl is used in photodynamic therapy in providing reactive oxygen species that are used in cancer treatment[12, 15, 16].

Our study aims to evaluate the hemocompatibility of the PcGaCl thin film. The cytotoxicity of the PcGaCl thin film will be also demonstrated in dark and with laser irradiation to Evaluate the effectiveness of synthetic films in PDT.

2. Materials and Methods

2.1. Deposition nanostructured PcGaCl thin films

PcGaCl powder has been purchased from Sigma-Aldrich Company. The thin film of PcGaCl was prepared by using an Auto HHV306 high vacuum coating system. At a pressure of 2.3 10. PcGaCl films were produced as previously described. The PcGaCl films were deposited on highly cleaned FTO layer/glass substrates with different thicknesses of 45, 75, and 100 nm. The thickness of PcGaCl was controlled by a quartz crystal monitor with a deposition rate of 3.5 nm/s.

2.2. Characterization of PcGaCl

2.2.1. UV Spectrophotometry

The optical spectroscopy measurements of PcGaCl thin films were done using the UV–Vis- spectrophotometer (JASCO, V-570). The measured parameters were estimated at a wavelength range of 200–1200 nm. Wemple–DiDomenico (WDD) for a single oscillator was utilized to calculate the energy gap of PcGaCl films.

2.3. Hemolytic Effect of PcGaCl Thin film.

Human blood samples were collected on Li-heparin as an anticoagulant. 2 ml of blood were centrifuged at 800xg and RBCs were isolated. Isolated RBCs were resuspended in autologous plasma at adjustable hemoglobin concentrations of 10 ±2 mg/ml. Blood aliquot was supplemented with PcGaCl thin films. Blood samples were incubated with PcGaCl thin film for 1 hr at 37 °C in the water path. After incubation blood samples were centrifuged at 800xg for 10 minutes and supernatant was used to determine hemolysis percentage. Hemolysis percentage was determined spectrophotometrically at 540 nm.

2.4. Effect of PcGaCl thin film on plasma coagulation time

blood samples were collected from male healthy volunteers with sodium citrate as an anticoagulant. Platelet Rich Plasma (PRP) was collected by centrifugation of blood at 800 x g for 20 min at 23°C. PcGaCl thin film (dimensions 5 mm × 5mm) was incubated with PRP for 1 hr at 37 °C in the water path. After incubation activated partial thromboplastin time (APTT) was measured. APTT was determined by mixing 0,1 mL of PRP with an equal volume of APTT-reagent and incubating for 5 minutes at 37°C in a water path. the coagulation was initiated by adding 0,1 mL of 0.025 M solution of $CaCl_2$. Clotting time was monitored by the coagulometer (HumaClot Junior - Germany).

2.5. Platelet Aggregation Study

0.5 mL of PRP was incubated with PcGaCl thin film (dimensions 5 mm × 5mm) for 1 hr at 37 °C in the water path. The thin film was then removed from PRP. 0.5 ml of PRP was then incubated with 0.02 mL of 0.025 M $CaCl_2$ and ADP in a final concentration of 12.5 μM at 37°C. Platelet Aggregation was monitored for 5 min using an aggregometer (APACT4004 LABiTec-Germany)

2.6. Dark cytotoxicity and In vitro PDT evaluation of PcGaCl thin film

Dark toxicity of PcGaCl thin film (In absence of Laser radiation) was evaluated using Caco-2, melanoma, and fibroblast. Cells density of 2 x 10^4 cells per well for each cell type were seeded on cells were seeded on the surface of the substrat**e.** 24-well tissue culture plates for 24, 48, or 72 h at 37°C in for 4 h in an atmosphere of 95% air and 5% CO_2. The cells plated on these substrates were incubated in 55 cm^2 culture dishes (Sigma-Aldrich, St. Louis, MO, USA). Cell

plates were wrapped in aluminum foil in a humidified incubator at 37 ″C with 95% air and 5% CO_2. The cells were grown to 80% confluence and dissociated with 2.5% trypsin (Gibco) at 37 °C for 2 min to obtain complete cell detachment. The cell suspension was centrifuged at 800 g for 5 min; after this, the supernatant culture medium was removed and the cell pellet was suspended with 4 mL of fresh culture medium. 1×10^6 cell/mL of cell suspension was plated on the control standard borosilicate coverslip. Each cell type was tested in triplicate. After incubation cells were washed twice with PBS. 500 µl of culture medium were mixed with100 µl of MTT (methyl-thiazolyl-tetrazolium) solution (1 mg/ml in PBS) in a plate well. After 4h incubation, the medium was removed and the resultant formazan crystals were dissolved in 500 µl of DMSO (dimethyl sulfoxide). Absorbance was measured for each well using a standard microplate reader at a wavelength of 490 nm with a reference wavelength of 620 nm. The relative cell viability (%) was calculated as the following:

$$Cell\ Viability\ \% = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

The previously explained procedure for cell viability was repeated with irradiated with a diode laser at a wavelength of 661 nm. The output power of the laser beam was 100 (Red) mW and, 200 (IR) mW. Cells were examined under the sequences of laser radiation of 2.5 J/cm^2, 4.5 J/cm^{2,} and 8.5 J/cm^2. All irradiations were performed at room temperature in the dark.

3. Results and discussion

For investigating the medical application of PcGaCl thin films, first, we have to study the optical properties using UV-spectrophotometer. The optical transmission, T, of PcGaCl thin films has been illustrated in Figure 1. According to Figure 1, the investigated thin film is transparent at a high frequency. At low frequency, the light absorption begins to appear. At 500 nm, an increase in our invastiagted thin film absortion. The absorption coefficient of PcGaCl thin films is calculated using:

$$\alpha = \frac{1}{d} ln\ ln\ \left(\frac{1-R}{T}\right) \quad (1)$$

where d is the sample thickness. Figure 2 shows the variation of α with the wavelength for PcGaCl thin film. The figure shows that the absorption of our investigated films has an optimum value of around 500 nm. We suggest this wavelength to be used in photodynamic therapy using PcGaCl as a senstizer. Furthermore, the optical band gap for PcGaCl thin film can be calculated using the following relation [17]:

$$(\alpha h\upsilon)^A = S\ (h\upsilon - E_g) \quad (2)$$

where S, $E_{g,}$ and A are the band tailing parameter, the optical energy bandgap, and the transition process constant, respectively. The values of A change due to the type of electrons transitions within the energy gap. For indirect allowed transition, A = ½, and the allowed direct transition A = 2. Figure 3 shows plots of $(\alpha h\upsilon)^{1/2}$ versus $h\upsilon$ for PcGaCl thin film. Regarding Eq. 3, the numerical value E_g can be estimated from the intercepts with the x-axis in both parts of Figure 3. The obtained value of the direct energy gap of PcGaCl thin film is 3.02 eV

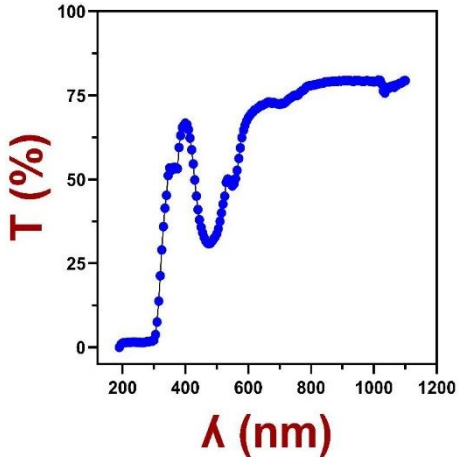

Fig. 1. Transmission of light through PcGaCl thin films.

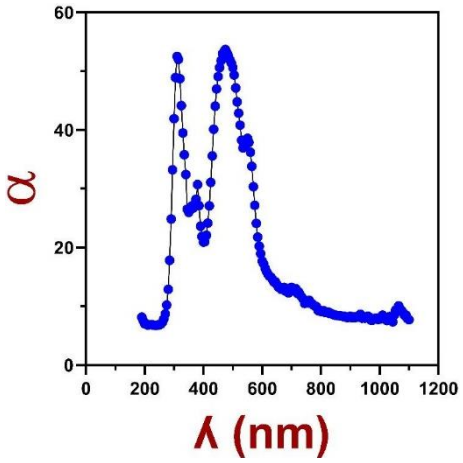

Fig. 2. light absorption within PcGaCl thin films.

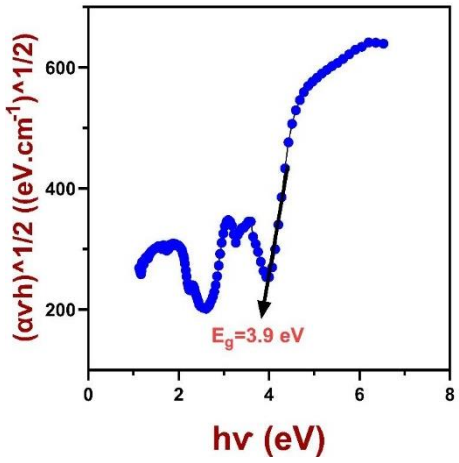

Fig. 3. the relation between $(\alpha h\gamma)^{1/2}$ and photon energy.

One of the most important biocompatibility tests is haemocompatibility. Haemocompatibility testing is to evaluate undesirable effects (e.g., hemolysis, thrombus formation, alterations in coagulation) in the blood, caused by a medical device or by chemicals

leaching from a device. Blood coagulation is one of the most hemocompatibility tests used in the assessment of the interaction between synthetic materials and blood. The interaction of thin film with blood coagulation proteins is a well-studied area[18-21]. Previously it was shown that carbon-based thin films improve hemocompatibility[22, 23]. Ti–O thin-film hemocompatibility, was studied and showed no thrombus formation[24]. This interaction between thin films and blood depends on consists in the geometrical relations between the thin film and the protein molecules of the blood. In the present study, the hemolytic effect of PcGaCl thin film was evaluated Figure 4. We found the nonhemolytic effect of PcGaCl thin film since the hemolysis percentage was less than 2%. The basic coagulation test APTT was performed in the presence of PcGaCl thin film. It was shown that PcGaCl thin film increased coagulation time in comparison with control (Figure 5) but the APTT was in the normal range. our experiments showed inactivating action of PcGaCl thin film on platelet aggregation (Figure 6). This could be caused according to the absorption of fibrinogen on the surface of the thin film that is sufficient for platelets aggregation.

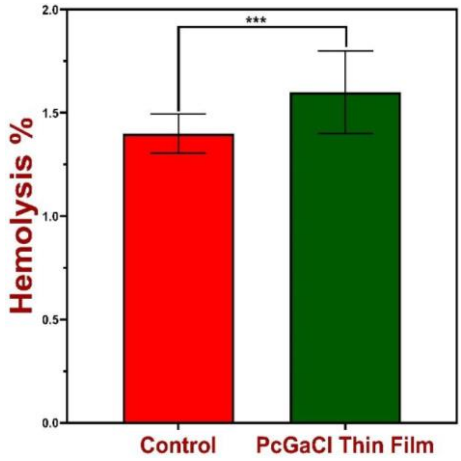

*Fig. 4. Hemolytic effect of PcGaCl thin film on human blood. *** Statistically significant difference ($p < 0.05$).*

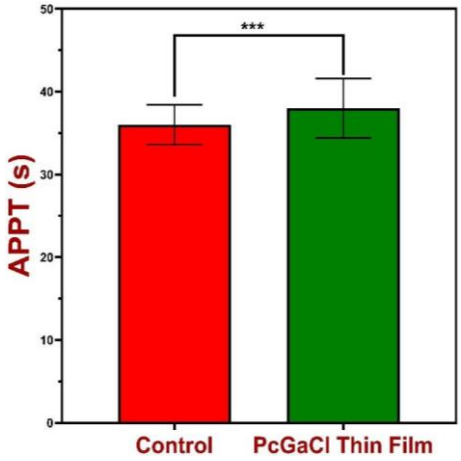

*Fig. 5. Activated partial thromboplastin time (APTT) of human blood plasma in the presence of PcGaCl thin film. *** Statistically significant difference ($p < 0.05$).*

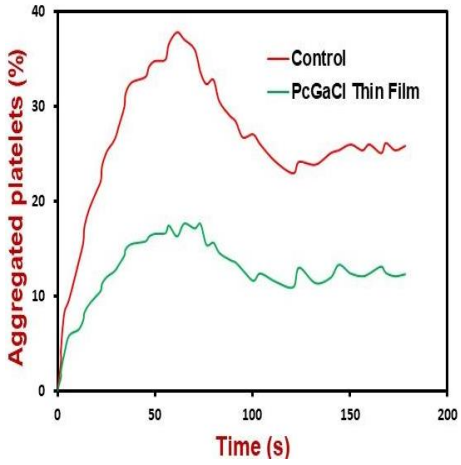

Fig. 6. Platelet aggregation in platelet-rich plasma (PRP) induced by PcGaCl thin film.

The cytotoxic effect of inactive PcGaCl thin film (dark treatment) on different cancer cell lines compared to healthy normal cell lines is presented in Figure 7. decrease in the cell viability was detected for cancer and healthy normal cells caused by incubation of the cells in the dark with PcGaCl thin film. for all cancer and healthy cells, the toxic effect of GaPcCl starts to appear after 24 h. The toxicity effect was gradually increased over time as shown in Figure 4. PcGaCl thin film was moderately toxic to Caco-2, melanoma, and fibroblast cells reducing cell viability to 70% or below after 72h.

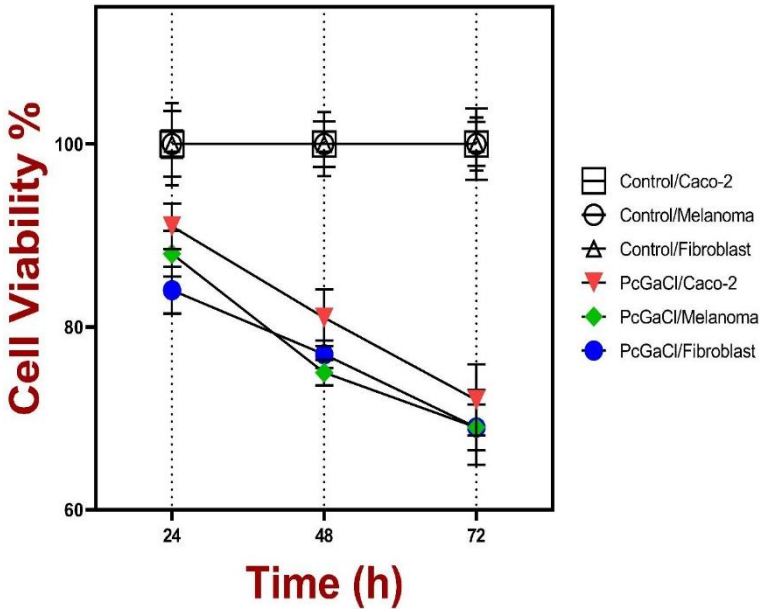

Fig. 7. cell viability at 24, 48, and 72 h after incubation with PcGaCl thin film. Error bars represent the standard deviation of the mean of three replicated experiments. (ANOVA) Analysis of variance showed that differences were significant ($p < 0.05$) between the cells incubated with PcGaCl thin-film ad its control.

PDT depends on o the accumulation of photosensitizer by cancer cells after the laser treatment stage [6]. The cell viability of cancer cells incubated with PcGaCl thin film after laser treatment was significantly reduced when compared to the untreated controls Figure 8. Control cells were exposed to the laser only to rule out the possibility that the laser is responsible for the destruction of the cells. Caco-2 cancer and melanoma cells were photosensitized with different

radiation doses of 2.5 J/cm^2, 4.5 J/cm^2, and 8.5 J/cm^2 as shown in Figure 5. At a 2.5 J/cm^2 cell viability was decreased to 77 %, 81% , 87% for Caco-2, melanoma, and fibroblast cells respectively (Figure 5). Further decrease in cell viability was monitored at 4.5 J/cm^2 and 8.5 J/cm^2 for all types of cells. Healthy normal fibroblast cells survived in vitro PDT treatment much better than the cancer Caco-2, and melanoma cells. This illustrates that PSs such as Pcs accumulate in cancer cells compared to normal healthy cells.

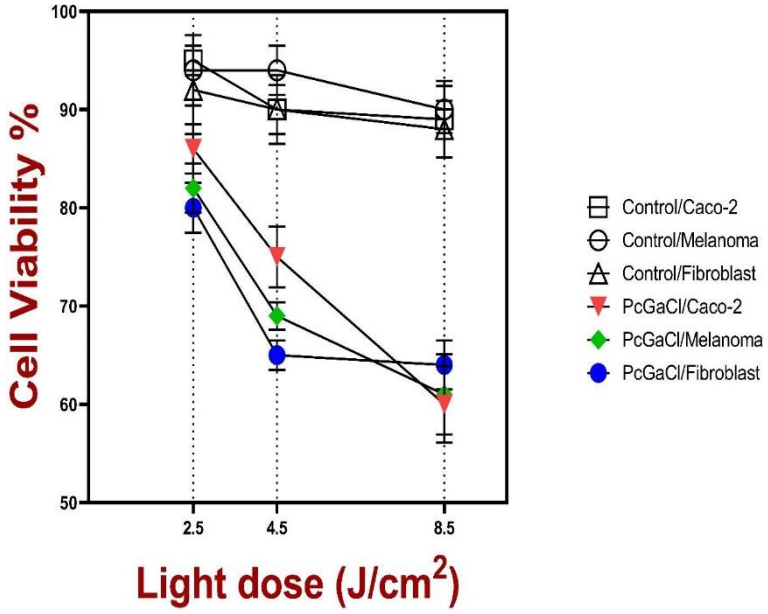

Fig. 8. Cell viability after irradiation with 2.5, 4.5, and 8.5 (J/cm^2) incorporated with PcGaCl thin-film incubation. Error bars represent the standard deviation of the mean of three replicated experiments. (ANOVA) Analysis of variance showed that differences were significant ($p < 0.05$) between the cells incubated with PcGaCl thin-film ad its control.

Studies showed that water-soluble cationic gallium(III) and zinc(II) phthalocyanines are not cytotoxic in the dark (IC50 > 100 mM) and localize intracellularly in multiple organelles, including the ER, Golgi apparatus, lysosomes, and to a smaller extent in the cell mitochondria. Upon exposure to a low light dose (1.5 J/cm2), 1Zn revealed high phototoxicity in human HEp2 cells. This is probably due to its enhanced ability for cellular internalization and generation of singlet oxygen within cells. We concluded that 1Zn could have the potential of photosensitizer for PDT[25]. Also, another study showed that The viability of Hepa-1C1C7 cells was reduced until 13× when the cells were irradiated in the presence of encapsulated GaPc while it was decreased until 4.7× when the experiment was carried out with the free GaPc. The encapsulated GaPc was also more efficient to cause the hemolysis of the RBC than it was the free GaPc. The optimization of the nanoparticles synthesis increased the efficiency of the GaPc to oxidize the evaluated cells[14, 26]. Another study has evaluated, Ga(III) Pc as a potential PS in neuroblastoma therapy. Results have shown that Ga(III) Pc has a good toxicological profile and PS characteristics for applicable PDT in the neuroblastoma cell lines. In addition, our results point out new mechanisms that should be further investigated as the PS localization and the long-term delayed decline effect post-PDT indicate complex intracellular pathways triggering tumor cell death.

4. Conclusion

Phthalocyanines (Pcs) are one of the most promising second-generation PSs and meet many requirements for being ideal PSs. GaPc can be a good PS because it has good non-toxic concentrations as proven by dark cytotoxicity tests. In our study, both hemocompatibility and cytotoxicity studies were done for PcGaCl thin film. We found the nonhemolytic effect of PcGaCl thin film since the hemolysis percentage was less than 2% while APTT was in the normal range and inactivating action of PcGaCl thin film on platelet aggregation. Also, there is a reduction of cell viability to 77 %, 81%, and 87% for Caco-2, melanoma, and fibroblast cells respectively in presence of different radiation doses of laser in comparison to cell viability in dark. PS's accumulated molecules will be activated upon irradiation with a particular wavelength of laser light inducing photochemical and photophysical processes. These processes reside in the energy transfer to the nearby oxygen, transfer that generates reactive oxygen species (mainly singlet oxygen, hydroxyl radical, and superoxide anions). As a consequence, oxidative stress induced by PDT is generated, cellular organelles and membranes become damaged, a process known as tumor photodamage.

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