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# The effect of silicon phthalocyanine on cell death and mitochondrial membrane potential in pancreatic cancer cells

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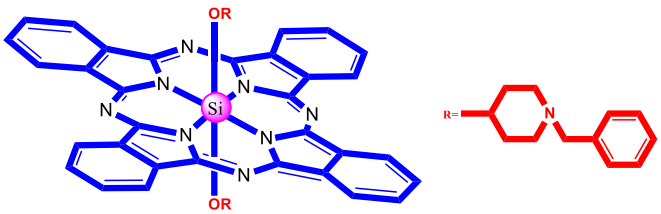
## Abstract

Silicon phthalocyanines (SiPcs) are advantageous inorganic molecules because they do not aggregate due to their special structural features. To deal with these structural obstacles, SiPcs have been widely used in a range of disciplines associated with chemical and biological technology. One of the common applications is the use of phthalocyanines as cytotoxic agents in cancer therapies. Cancer is the disease threatening human life and reducing life span and quality, and pancreas cancer is one of the most aggressive cancers with a high rate of mortality around the world. In this study, we therefore aimed to investigate the potential effects of SiPc molecule synthesized for the first time in our previous study on cytotoxicity and mitochondrial activity on pancreatic cancer cells. The results showed the significantly selective cytotoxic effect of SiPc (with a high selective index as 2.5 – 5) on cancer cells compared to normal cells. Mitochondrial membrane potential was not different in cancer cells after SiPc treatment, but interestingly mitochondrial membrane potential of normal cells significantly changed after the treatments. Pre-incubation time (24h) of SiPc before light irradiation induced more significant cytotoxicity in pancreatic cancer cells but not in normal cells compared to prolonged pre-incubation (48h). This study revisited the biological function of previously synthesized SiPc, and the results conclude the cytotoxic activity of SiPc on pancreas cancer. Findings in this work can be extended for other cancer types and detailed with in vivo models in the future.

**Keywords:** Silicon phthalocyanine, cancer, cytotoxicity, mitochondrial membrane potential

## 1. Introduction

Phthalocyanines (Pcs) area large family of hetero-cyclic conjugated compounds with strong chemical stability. These characteristics make phthalocyanines a significant structural material that can be utilized in many applications such as systems of optical data storage, gas sensors, switching devices and photosensitisers in medical applications [1–5]. One limitation of Pcs applications in solutions is that phthalocyanines are characterized by a very high tendency to aggregate, which reduces their photosensitizing ability through self-quenching. The aggregation of phthalocyanines results in some difficulties in purification and characterization. The lowering of aggregation behaviour can be achieved by the bulky groups to the central metals which have more than four coordination numbers. Axially substituted phthalocyanines are much less likely to aggregate because of steric hindrance of the non-planar substituents to the central atom [6–10] that is why axially 1-benzyl-4-oxy units substituted silicon phthalocyanines has been chosen in this work (Fig. 1).




SiPc

**Figure 1.** Axially 1-benzyl-4-oxy substituted silicon phthalocyanine

In our previous work, we investigated electrochemical, photocatalytic and aggregation behaviour of 1-benzyl-4-oxy substituted silicon highly soluble in most of the organic solvents and are investigated aggregation

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behaviour in different common organic solvents. The photocatalytic activity of SiPc 3 was examined for *p*-nitrophenol degradation using photochemical reactor. The results indicated that the catalyst showed good activity for *p*-nitrophenol degradation to the corresponding hydroquinone as major product in 1h at 25 °C. Voltametric studies suggested that SiPc displays reversible/quasi reversible/irreversible redox processes, which are the main requirement for the technological usage of this compound [11].

Cancer is one of the most serious health problems worldwide with high mortality. One of the cancer types with the lowest survival rate is pancreatic cancer. Pancreatic ductal adenocarcinoma (PDAC) is the sub-type observed in more than 90% of patients [12]. Phthalocyanines and their derivatives are the molecules with strong absorption in near-infrared regions, chemical and thermal stability, and low toxicity in the dark, in general use to cancer therapy [13]. Phthalocyanines are used as individual cytotoxic agents with the high selectivity for cancer cells [14] or combined with other drugs [15].

Mitochondrion is an organelle serving as the main producer of ATP, and the metabolites necessary to produce macromolecules and reactive oxygen species (ROS). Abnormalities in mitochondrial function and oxidative stress have been associated with the pathologies of some diseases. Mitochondrial membrane potential ($\Delta\psi m$) is a parameter indicating the proper physiology of the cell. A shift of energy metabolism from oxidative phosphorylation to active glycolysis and an increase in the generation of reactive oxygen species are observed in many cancer cells. These metabolic changes are usually associated with the formation of NAD(P)H oxidase. Mitochondria-dependent metabolic reprogramming in cancer cells is associated with oncogenic signals [16]. In this study we aimed to reveal the cytotoxic effect of our previously synthesized silicon phthalocyanine molecule as well as the potential effect on mitochondrial membrane potential in pancreatic cancer cells.

## 2. Experimental

### 2.1. Materials

SiPc was designed and prepared in our previous work (Fig. 1) [11]. All solvents were dried and purified as described by the reported procedure [17]. 4-Nitrophthalonitrile was purchased from commercial suppliers.

AR42J pancreas cancer (ATCC, Cat No CRL-1492™) and Sol8 normal myoblast (ATCC, Cat No CRL-2174™) cell lines were cultured in RPMI and DMEM media, respectively. All complete media included 20% FBS and 1% penicillin/streptomycin.

The light source used was red light at a wavelength of 680 nm at an energy density of 10 j/cm$^2$.

### 2.2. Methods

After cells reached full confluency (so the cells were almost 100% confluent on culture vessel), cells were treated with different concentrations of SiPc at 1.5, 3, 6 and 12 µM SiPc. Upon treatment with SiPc at 24h or 48h, cells were exposed with 680 nm light for 1 hour at room temperature. Some cells were not treated with light, therefore 4 experimental groups included are as follows:

Group 1; 24h incubation followed by light exposure
Group 2; 24h incubation followed by dark exposure
Group 3; 48h incubation followed by light exposure
Group 4; 48h incubation followed by dark exposure

The day after light exposures MTT assay and mitochondrial membrane potential analyses were performed.

#### *2.2.1. Activation of SiPc with light in cells*

PDT was applied according to the formula; J = W x S (J = desired amount of light energy, W = Light power received by the sensor and S = Time period (hour) to be applied depending on desired light energy and light power). Therefore, cells were treated with red light at a wavelength of 680 nm at an energy density of 10 j/cm$^2$ for 1 hour. After light activation cells were incubated at 37 °C with 5% $CO_2$ for further 24h.

#### *2.2.2. MTT method*

After drug treatments and light exposures, media was removed, and cells were washed with 100 µl PBS containing $Ca^{2+}$ and $Mg^{2+}$. 10 µl of MTT and 190 µl of complete media were added to each well, and the cells were kept at 37 °C for 2 hours. After 2 hours, media containing MTT was withdrawn, and 200 µl of DMSO was added. Plates were incubated on a shaker overnight at room temperature followed by reading absorbances at 570 nm in the spectrophotometer [18].

#### *2.2.3. Mitochondrial membrane potential ($\Delta\psi m$)*

After drug treatments and light exposures, media was removed, and cells were washed with PBS containing 100 µl of $Ca^{2+}$ and $Mg^{2+}$. Cells in each well were incubated in 200 µM complete media containing 400 nM Mitotracker red dye, at 37 °C for 45 minutes, followed by

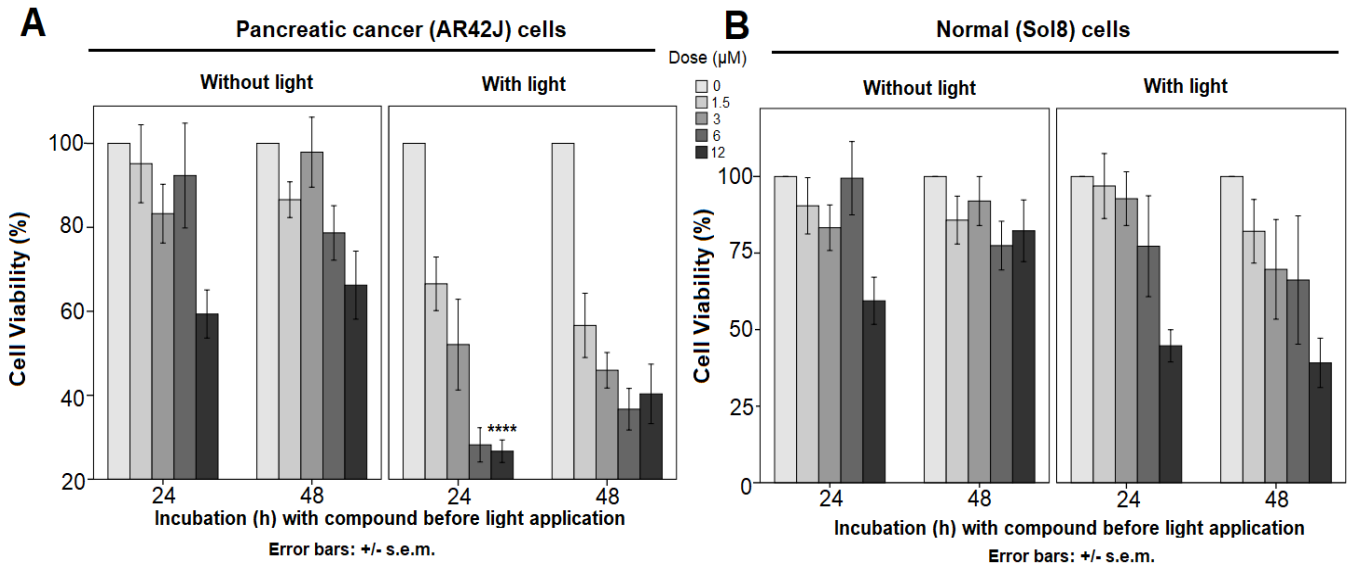


**Figure 2.** A) Cell viability of pancreatic cancer cells (AR42J) after SiPc and B) cell viability of normal muscles cells (Sol8) after SiPc

reading fluorescence at a wavelength of 579 – 599 nm the spectrophotometer [18].

*2.2.4. Statistical analyses*

Cell viability (%) and mitochondrial membrane potential were analysed by UNIANOVA (univariate analysis of variance) using SPSS software, Version 23. Data was shown with +/- standard errors of the means of experimental repeats (including inter- and intra-repeats). $p$ values less than 0.05 were considered as significant.

## 3. Results and discussion

SiPc treatments both for 24h and 48h only after light exposure induced cell death in pancreatic cancer (AR42J) cells ($p < 0.0001$) (Fig. 2A). Although the lowest dose (1.5 μM) induced cell death on cancer cells (after light), the highest dose (12 μM) only induced significant cell death on normal cells after light (Fig. 2B). However, there was no significant difference between incubation time with drugs before light exposure ($p > 0.05$). Consistently, $IC_{50}$ values were more in Sol8 normal cells than in AR42J pancreatic cancer cells after light exposure (Table 1). SiPc had a cytotoxic effect on cells even if no light activation, but this effect was rather low (Table 1). Phthalocyanines are advantageous as they are applied locally to be activated by light irradiation. This local activation leads to specific targeting of cancer cells [19]. In this study SiPc showed a selective cytotoxic effect on cancer cells with selective indexes around 2.5 – 5 in treated cells at 24h before light application (Table 1). PDT has been shown to selectively affect cancer cells with increased cytotoxicity [20–22]. Cancer is a curable abnormality but one of the main drawbacks of current cancer therapies is low selectivity of cytotoxic agents therefore the treatment may be harmful for non-cancerous cells by inducing cell death or differentiation mediated by new mutations in normal cells [23]. This handicap is overcome by the discovery of selective cytotoxic agents which affect cancer cells only at the applied conditions. These advantageous agents are supposed to target cancer-specific molecular changes.

Another limitation in drug design is the solubility properties of the candidate compounds. Almost all organic and inorganic compounds are dissolved in dimethylsulfoxide (DMSO) and ethanol but especially the high doses of DMSO itself can trigger toxicity in the cells. Therefore solubility is preferred in safety drug applications and many methodologies are used to enhance the solubility of the compounds [19,24–28] and the SiPc molecule examined in this study was highly soluble therefore it can be considered as solvent-safe for the cells. But these results should be extended for detailed investigation using mice models and other molecular techniques. The used SiPc can be also substituted with other drug molecules, such enzyme inhibitors [29]. Zinc and silicon Pcs were previously shown to have cytotoxic effects on cholangiocarcinoma, the second common type of hepatic cancer [30].

Mitochondrial membrane potential (MMP) ($\Delta\psi m$) reflecting the functional effectiveness of mitochondria is a biomarker in cell death which tends to decline in

**Table 1.** $IC_{50}$ values after SiPc treatments in AR42J and Sol8 cells and selective indexes

| | | Average$IC_{50}$(μM) | |
|---|---|---|---|
| **Cell Line** | **Pre-Incubation (h)** | **+ Light** | **- Light** |
| **AR42J** | 24 | 3.54 | 23.30 |
| | 48 | 1.98 | 20.75 |
| **Sol8** | 24 | 17.52 | 22.20 |
| | 48 | 4.95 | 209.53 |
| **Selective index (SI)** | 24 | 4.94 ± 2.73 | 0.95 ± 0.22 |
| | 48 | 2.5 ± 0.78 | 10 ± 5.41 |

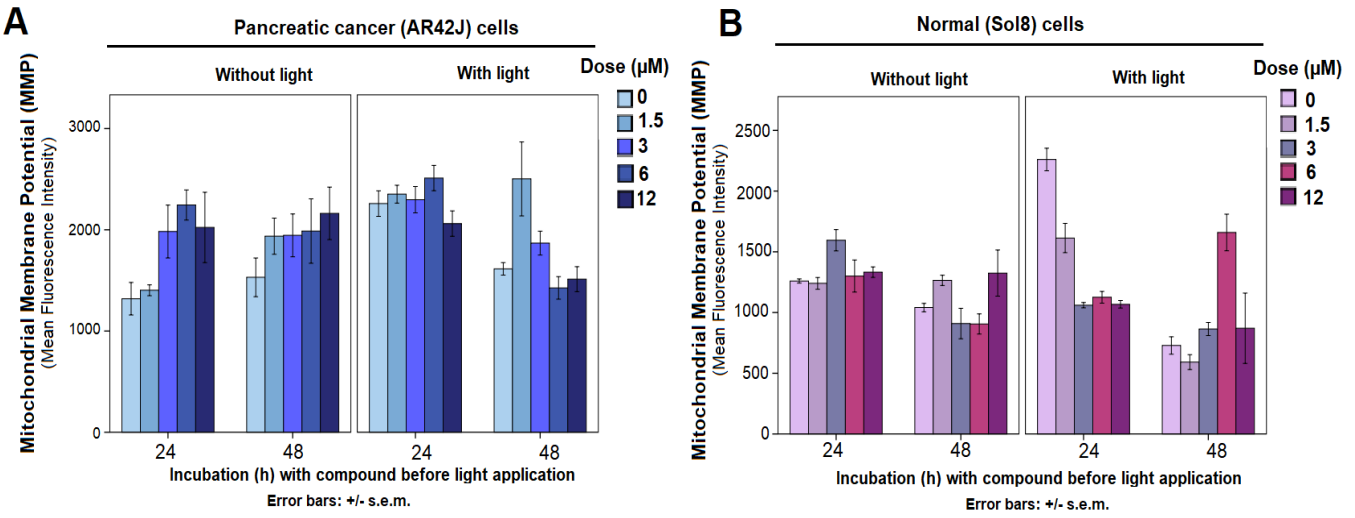


**Figure 3.** A) Mitochondrial membrane potential in pancreatic cancer cells (AR42J) after SiPc and B) mitochondrial membrane potential in normal muscles cells (Sol8) after SiPc

concordance with apoptosis. MMP has been shown to be highly positively associated with cancer malignancy. Especially cancer stem cells have mitochondrial metabolism variability and targeting mitochondria therefore come forward in cancer research [31]. It has been shown that targeting mitochondria increased the effect of photodynamic therapy [32]. Mitochondria are the organelles where oxygen levels are high so that reactive oxygen species can be easily produced, and apoptosis is commonly initiated. This suggests that an ideal photodynamic therapy agent could be a potent photosensitiser that can naturally accumulate in mitochondria. Therefore, the capacity of the new phthalocyanine molecule to be used in this study to target mitochondria was investigated. Some nanoparticles were shown to induce both cell death and the loss of MMP [33]. Hyaluronic acid formulated nanoparticles containing a platinum(II) conjugated silicon(IV) phthalocyanine (SiPc-Pt-HA) were shown to accumulate in mitochondria of MDA-MB-231 breast cancer cells compared to non-cancerous cells [34]. In this study, MMP was not significantly changed in cancer cells at 24h (Fig. 3A) but decreased in normal cells (Fig. 3B). In principle, during PDT the molecular oxygens within the cell are converted into singlet (stimulated) oxygen by stimulating the cytotoxic agent, the photosensitiser, after light applied at the appropriate wavelength. Therefore, mitochondrial membrane potential is supposed to be changed. Normal cells used in this study were muscle cells, and muscle cells are a type of cells with a high number of mitochondria. Therefore, Sol8 cells might be more sensitive to the used silicon phthalocyanine in terms of mitochondrial function. Nevertheless, both types of cells treated with SiPc for prolonged incubation (48h) before light activation did show inaccurate changes on MMP.

## 4. Conclusion

In conclusion, photosensitiser potential of axially 1-benzyl-4-oxy units substituted silicon phthalocyanine on cell death and mitochondrial membrane potential in pancreatic cancer cells was investigated. Our previous work reported that SiPc used in study is highly soluble and has a photocatalytic activity. In this work, previously synthesized SiPc was investigated in terms of cancer therapeutic potential, and it was shown that SiPc had a selective cytotoxic effect (with high selective indexes from 2.5 to 5) but not significant effect on mitochondrial activity on pancreas cancer cells. Normal muscle cells were more sensitive to SiPc in terms of the changes in MMP than normal cells due to most probably their ontological mitochondrial content. Lower pre-incubation of SiPc before light resulted in higher rate of cell death than longer incubation. These results indicate the anti-cancer activity of SiPc, but its molecular mechanism of action should be detailed by extensive molecular and cellular methods.

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