Effect of Photon Radiations in Semi-Rigid Artificial Tissue Sensitized by Protoporphyrin IX Encapsulated with Silica Nanoparticles

Ghaseb N. Makhadmeh1, 3, 4, *, Azlan Abdul Aziz1, 3 and Khairunisak Abdul Razak2, 3, M-Ali H. Al-Akhras5

1School of Physics, Universiti Sains Malaysia, Penang, Malaysia.
2School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, Penang, Malaysia.
3 NanoBiotechnology Research and Innovation (NanoBRI), Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia.
4Arts and Science College, Amman Arab University, Amman, Jordan
5Bio-Medical Physics Laboratory, Department of Physics, Jordan University of Science & Technology, Irbid, Jordan

ghaseb84@yahoo.com

Abstract: This study involves the synthesis of Protoporphyrin IX (PpIX) encapsulated with Silica Nanoparticles (SiNPs) as an application for Photodynamic therapy. Semi-rigid artificial tissues with optical features similar to human tissue were used as sample materials to ascertain the efficacy of PpIX encapsulated with SiNPs. The disparity in optical characteristics (transmittance, reflectance, scattering, and absorption) of tissues treated with encapsulated PpIX and naked PpIX under light exposure (Intensity at 408 nm ~1.19 mW/cm²) was explored. The optimal exposure times required for naked PpIX and SiNPs encapsulated PpIX to engulf Red Blood Cells (RBCs) in the artificial tissue were subsequently measured. Comparative analysis showed that the encapsulated PpIX has a 91.5 % higher efficacy than naked PpIX. The results prove the applicability of PpIX encapsulated with SiNP on artificial tissue and possible use on human tissue.

Keywords: Photodynamic Therapy, Silica Nanoparticles, Encapsulation, Protoporphyrin IX, Optical Properties, Artificial Tissue.

1. Introduction

The current advancement in Photodynamic Therapy (PDT) has offered new medical approaches to cancer treatment, such as the use of photosensitizers (PSs) under light exposure of a specific intensity [1]. Singlet oxygen \(^{1}\text{O}_2\) produced by the light exposure engulfs the cells by means of cytotoxicity [2-4]. However, the efficiency of \(^{1}\text{O}_2\) production decreases significantly because of the small volume of PS that reaches the target cells. This decline in PS efficacy is attributable to the defense mechanism of reticulo-endothelial system (RES) or the engulfing of PS by the macrophage system in the body. Nonetheless, macrophage normally gives no attention to biocompatible foreign objects with sizes under 100nm [5]. Thus, the inhibition effect of macrophage can be resolved using nanoparticles as a medium of PS transportation. This study proposes the use of silica nanoparticles (SiNPs) as an encapsulating medium for PS. The intrinsic features of SiNPs such as low toxicity and high biocompatibility ensures its effectiveness as an ideal encapsulation material for Drug Delivery System (DDS) [6]. In addition, it is easily synthesized with low polydispersity at low temperatures. Moreover, bio-molecular compounds have a propensity to agglutinate to the external surface of SiNPs, while its inner surface has the distinctive capability of encapsulating PS [7-9].
Protoporphyrin IX (PpIX) is an essential derivative of porphyrin [10-12]. The absorption of emitted energy light source by PpIX leads to a series of chemical reactions which generate singlet oxygen. The singlet oxygen produced is capable of engulfing the artificial tissue [13-16]. Therefore, this study evaluates the effect of PpIX encapsulated by SiNPs on Red Blood Cells (RBC’s) using artificial tissue (light absorption component) as test sample [17-18]. The transmittance, reflectance and optimal exposure time properties of the test sample were determined. Variations in absorption, scattering coefficient, the relation between the depth and the transmittance and Half Value Layer (HVL) of the RBC’s were also analyzed. The efficacy of encapsulated PpIX on RBCs destruction was then compared with that of naked PpIX.

2. Materials and Methods
SiNPs were prepared using a microemulsion method [19]. The first step involves mixing 0.1ml of ammonia (Sigma Aldrich Cat No: 1336, 21, 6), 200ml distilled water, and 5.5g Tween 80 (Sigma Aldrich Cat. No. P1754). The solution was constantly stirred for 15 min. The measured pH value was found to exceed 9.0. A 6 ml of 1-Butanol (Sigma Aldrich Cat No: B8, 590) was subsequently added, and the solution stirred for further 5 minutes at room temperature. The next step entails the encapsulation of 15 ml PpIX (10.04 μM as a final concentration). Following the encapsulation, the sample was wrapped in aluminum foil because of its high sensitivity to light. After 1 hour, 2 ml Triethoxyvinylsilane (TEVS) (Sigma Aldrich Cat. No. 175560) was added, and the solution was continually stirred at a temperature of 27°C for 20 hours. After carrying out the sample preparation, dialysis was performed on the solution using a dialysis membrane (by soaking the dialysis membrane in distilled water for 5 min) [18].

100 ml of artificial tissue was prepared by dissolving 2 grams of agar (No. 9405, Sigma Aldrich) in 40 ml distilled water and 40 ml of the Phosphate Buffered Saline (PBS) (1 tablet in 200 ml distilled water, No. P4417, Sigma Aldrich). The temperature of the solution was increased to 100°C for 3 min. The sample was stirred continuously during the course of heating up and cooling down. Following the drop in temperature to less than 80°C, 3gm of 5-10μm diameter silica powder was added. The solution was subsequently mixed for 5 min. A 1 ml of solution ink (Abs. equal to 0.18 at 500 nm after diluting for 100 times), 20 ml of bovine serum and 0.2 ml of intralipid were incorporated into the solution at 45°C. 1.5 ml of concentrated blood (Abs. equal to 0.90 at 500 nm after diluting for 100 times), 10.04 μM PpIX encapsulated with SiNPs, and 1 ml of penicillin were also added at 37 °C.

The resultant solution was poured into sample holders containing semi-rigid artificial tissue slices of 0.71 mm thickness. Afterward, the solution was stored overnight at temperatures below 4 °C. The next day, 5 sample slices were exposed to light (intensity at 408 nm ~1.19 mW/cm²) provided by an arc lamp positioned 30cm away from the sample. The experiment was carried out at various exposure times (0, 10, 25, 45 and 70 min.). The transmittance (T) and reflectance (R) of the samples were measured using NIR-UV-VIS Spectrophotometer. The scattering and absorption coefficients spectrums were derived using the Kubelka-Munk theory [19]. Different parameters were correlated, and such as: thickness of the sample and transmittance; thickness of the material at which the intensity decreased by one half (Half Value Layer (HVL)), and the optimal exposure time that is required to engulf half of RBCs. A similar sequence of measurements was also applied on naked PpIX.

3. Results and Discussions

3.1. Impact of Naked and Encapsulated PpIX on the Optical Properties of An Artificial Tissue
In order to determine the efficacy of encapsulation, the effects of using encapsulated PpIX were compared with those of naked PpIX. The difference in optical properties for both treatments was examined. As indicated by the encircled cusps in Fig. 1 (a) and (b), variations in minimum point of reflectance after light exposure were observed for samples treated with encapsulated PpIX and naked PpIX, respectively. For the sample treated with encapsulated PpIX, a comparably slightly higher increase in reflectance is observed at the minimum point of 577nm (maximum peak of RBCs absorption), possibly due to enhanced light energy absorption by encapsulated PpIX. This absorption process produces more 4O2 which increases the destruction of RBCs in the tissue.
Shifts in the spectrum are also observed in the minimum points of transmittance for both samples treated with encapsulated PpIX and naked PpIX as shown in Fig. 2 (a) and (b), respectively. The increase in minimum point of transmittance (577 nm) is attributable to the singlet oxygen produced by PpIX, which engulfs the RBCs in the sample, consequently increasing the transmittance value.

This trend is also evident in the scattering values at 577 nm as depicted in Fig. 3 (a) and (b). This is due to the fact that the singlet oxygen generated from PpIX light energy absorption engulfs the RBCs in the sample.

Figure 1. Changes in reflectance spectrums of the artificial tissue: (a) treated by encapsulated PpIX, (b) treated by naked PpIX.

Figure 2. Changes in transmittance spectrums of the artificial tissue: (a) treated by encapsulated PpIX, (b) treated by naked PpIX.

Figure 3. Changes in scattering spectrums of the artificial tissue: (a) treated by encapsulated PpIX, (b) treated by naked PpIX.
However, a contrasting observation was made for the absorption values, as shown in Fig. 4 (a) and (b). Rather, a decline in the maximum peak of absorption coefficient was noted. This is because the singlet oxygen, generated from the absorption of light energy by PpIX, engulfed the RBCs membrane, resulting in the release of hemoglobin in the sample which reduced the absorption peak.

![Figure 4](image-url) Changes in absorption spectrums of the artificial tissue: (a) treated by encapsulated PpIX, (b) treated by naked PpIX.

The higher decrease in the absorption coefficient of samples treated with encapsulated PpIX indicates a relatively faster rate of RBCs destruction compared to naked PpIX treatment because the agglomeration of encapsulated PpIX molecules by the SiNPs membrane enhances light energy absorbance and singlet oxygen production in contrast to the naked PpIX which is dispersed in the sample.

### 3.2 Optimal Exposure Time Measurement

This section entails the measurement of the optimal exposure time required for the samples (RBCs) to achieve half life time. Fig. 5 (a) depicts a decrease in the K’s maximum peak of the samples treated with naked and encapsulated PpIX, following the exposure to light source. This is due to the engulfing of RBCs by $^{1}O_{2}$ produced through PpIX absorption of light energy [21]. This phenomenon is also indicated by the decrease in absorption coefficient. For the sample treated with encapsulated PpIX, an abrupt drop in K’s maximum peak is observed in the first 10mins of light exposure, followed by a more subtle gradual decline, which indicates an initial rate of rapid RBC destruction. In contrast, treatment with naked PpIX show a steady decline in K’s maximum peak for the first 50mins of light exposure, accompanied a faster decline, which indicates the naked PpIX takes a longer time to take effect. The relatively higher decline in the K’s maximum peak of encapsulated PpIX treatment signifies RBCs are engulfed faster in the sample treated with encapsulated PpIX compared to naked PpIX.

Values of 5.78 and 68.00 min. were obtained for half life time of RBCs (fractional haemolysis of 50%) of samples treated with naked and encapsulated PpIX, respectively, as shown in Fig. 5 (b). The higher value for encapsulated PpIX is possibly attributable to the clustering and agglomeration of encapsulated PpIX molecules by SiNPs membrane, also pointed out by the increased absorbance of light energy and higher volume of singlet oxygen produced. For naked PpIX molecules, the molecules are homogeneously dispersed in the sample, which initiates a relatively slower interaction during exposure to a light source.
3.3 Correlation of Transmittance with the Thickness of Artificial Tissue
The variations in transmittance at 577nm as thickness (D) of the artificial tissue increases were measured for five samples, as shown in Fig. 6 (a). The Half Value Layer (HVL) of the samples was determined to be 0.45 and 0.53 mm for samples treated with encapsulated and naked PpIX respectively. The equation denoting the relationship between Transmission (T) and thickness (D) of the samples was derived from their Logarithm relation as shown in Fig. 6 (b).

![Figure 5](image1)

**Figure 5.** (a) Maximum peak of K vs. exposure time, (b) Optimal exposure time at 50 % of fractional haemolysis.

![Figure 6](image2)

**Figure 6.** (a) Relation between Transmission (T) and Depth (D), (b) Logarithm relation between Transmission (T) and Depth (D).

An evaluation of the HVL values for both samples showed no significant disparity between the samples treated with encapsulated and naked PpIX. It can be deduced from the results that the relationship between Transmission (T) and Depth (D) can be applied to determine the HVL for other samples without having to replicate experiments.

4. Conclusion
It can be inferred from the results that encapsulation considerably improves the efficiency and applicability of PpIX as a photodynamic agent in the treatment of artificial tissues compared to the naked PpIX. As demonstrated in the in-vitro studies above, encapsulated PpIX destroys more RBCs than the naked PpIX. In general, SiNPs exhibited a high efficacy of 91.5 % (percentage between t50 for naked and encapsulated PpIX).

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