# Resin phantoms as skin simulating layers

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**Abstract**. In order to apply light treatment to skin, the absorption through the outer layers of the skin needs to be considered. Darker skin has a higher concentration of melanin in the epidermis and absorbs more light than fair skin. Ideally the effect of the skin treatment on the outer layers of the skin should be tested on *in vitro* multi layer skin models. This is not always feasible. For this work, phantoms were used together with skin cancer cells to test the effect of outer layer absorption on the efficiency of Photodynamic Therapy (PDT) treatment. Two resin based solid phantoms were prepared to simulate two different skin types. Cells were prepared and PDT treatment were done on cells with and without the phantoms, by keeping the total dose delivered to the cells constant at 4.5 J/cm$^2$. Cell viability for the cells with the phantoms was less than without the phantoms and the differences are attributed to more uniform light distribution, but this needs to be investigated in more detail. The initial results of the experiments indicate that solid resin based phantoms can be used to optically mimic the effect of the outer skin layers.

## 1. Introduction

Lasers as both a research and application tool have been established in numerous fields in the last 50 years. The use of lasers in medical applications did not stay behind the other fields. As early as 1964, the ophthalmological applications of lasers were reported on [1].

Human skin is a highly scattering medium and the melanin in the epidermal layer of the skin is a major absorber of light in the visible and near infrared wavelengths. The human skin consists of several layers. In text books on light propagation through tissue [2], four layers are specified: stratum corneum (SC), epidermis, dermis and hypodermis.

The SC also called the horny layer consists of dead cells while the epidermis consists of melanosomes and melanocytes which produce the melanin in the skin that is responsible for skin colour. Most non-invasive laser treatment or diagnostic procedures need to penetrate the outer skin layers (SC and epidermis) to reach the treatment site. The melanin in the epidermis makes it a highly absorbing layer.

Melanin is an optically dense material which absorbs radiation in the visible wavelength range. Melanin is not a single pigment, but is composed of a number of different chromophores with varying optical and physical properties [3].

Darker skin has a higher concentration of melanin in the epidermis and absorbs more light than fair skin. Ideally the effect of the skin treatment on the outer layers of the skin should be tested on *in vitro* multi layer skin models before treatment. This is not always feasible.

PDT is a treatment where a photosensiteser (PS) or drug is applied to the cancer cells and in the presence of light, tuned to the absorption wavelength of the PS, and the oxygen present in the cells,

singlet and triplet oxygen are formed. The singlet and triplet oxygen is considered to be reactive oxygen species (ROS) that are lethal to the cells. The treatment is a localized treatment due to the short lifetimes of the singlet and triplet oxygen. Photosense®, which is a mixture of sulfonated aluminium(III) phthalocyanines, is clinically used in Russia for the treatment of a range of cancers [4].

In this paper the suitability of using solid skin simulating phantoms to mimic the effect of the outer skin layers were investigated by testing the efficacy of Photodynamic Therapy (PDT) treatment on cancer cells with and without the phantoms.

## 2. Materials and Methods

In this section the phantom preparation, the computer model used to predict the transmission through the sample and the cell work will be discussed.

### 2.1. Skin simulating phantoms

Skin simulating phantoms were prepared according to the recipe of Firbank [5,6]. Scattering particles (TiO particles) and absorbing particles (Carbon Black) were added to an optically clear resin base (Akasel) and a hardener (Aka-cure slow, Akasel). The mixture was poured into plastic containers with a diameter of 30 mm and was allowed to cure at room temperature under a fume hood for 24 hours.

The cured solid phantoms were cut into three 1 mm thick discs (A_1(I)-A_1(III)) to have a phantom for each of the 3 wells with cells that will be irradiated. The amount of carbon black was varied while the TiO concentration was kept constant. The ratios (per weight) were 133:0.25:31000 (TiO:carbon-black:resin) for the A_1 samples and 133:0.75:31000 for the A_3 samples. Both the absorption coefficient, $\mu_a$, and reduced scattering coefficient, $\mu_s'$, were measured with the integrating sphere (IS) system described in [7]

### 2.2. Computer modelling

Modelling the interaction of laser light with human tissue is an area of research in many parts of the world. Accurate modelling allows the researcher to test different extremes without the cost of clinical trials, even though it can never replace the value and importance of clinical trials. A model was developed in the ASAP software environment and tested to determine the accuracy and validity of the model. In the model the optical properties, $\mu_a$, $\mu_s'$, refractive index (n=1.4) and the anisotropy (g=0.79), are described for each layer or medium. A light source is specified and 3.1 million photons are traced, making use of a Monte Carlo process, through the system. The system is divided into thin layers (nominally 0.1 mm) for analysis. The number of photons absorbed and passing through each layer is calculated and stored for later analysis. More details can be found in [6].

The optical properties of the phantoms were measured with the IS system, the results were used in the computer model to predict the energy fluence at the bottom part of the model (light that was transmitted through the sample). More detail on this study at $\lambda$ = 632.8 nm can be found in [8].

### 2.3. Cell tests

For this paper Photosense®, commercially available and approved photosensitizer for some skin cancers, was obtained from the Chemistry Department of Rhodes University (courtesy of Prof T Nyokong). Stock solutions of 1 mg/ml sensitizer was made up in Dulbecco's modified Eagle's medium, (DMEM) with phenol and sterilized by filtration using a 0.2 µm filter.

#### *2.3.1. Cell culture*

Cultures of human squamous cell carcinoma cell line (A431 cell-line, obtained from Sigma-Aldrich, South Africa, Cat # 85090402) were grown in the supplemented culture medium (Dulbecco's modified Eagle's medium, (DMEM) with L-glutamine and phenol red, supplemented with 10 % heat-inactivated fetal calf serum (FCS) and 1 % penicillin-streptomycin mixture, 5K/5K) obtained from Whitehead Scientific (Pty) Ltd, South Africa, at 37 °C and 5 % $CO_2$.

*2.3.2. Cytotoxicity test*

The optimal concentration of Photosense® and diode laser dose were determined by incubating the cells with various concentrations of the photosensitizer (10, 20, 40 and 80 µg/ml) for 24 h or irradiating cells with 676 nm diode laser with a power density of 39,1 mW/cm$^2$ and irradiation at 0.5-5 J/cm$^2$ for 12,8-127.9 s, respectively. The laser used was build at the NLC from commercially available parts. The laser beam from the diode had a rectangular shape and was shaped through a lens to a square and an iris was used to deliver a round beam on the cells with an area of 1.2 cm$^2$ and a total power of 49.2 mW.

Cells were seeded into 24-well tissue culture plates at a density of 50 cells/mm$^2$ per well in 1 ml of supplemented DMEM with phenol red. Cells were allowed to attach for 48 h before being washed twice with 2 ml Dulbecco's phosphate buffered saline (DPBS, from Whitehead Scientific (Pty) Ltd, South Africa,) then photosensitized by the addition of culture medium containing 10, 20, 40 and 80 µg/ml Photosense®. Control cells contained medium without Photosense®. Plates were incubated at 37 °C in 5 % $CO_2$ in the dark for 24 h, the wells were then washed twice with 2 ml DPBS and the medium replaced with 1 ml of culture medium. The loss of sensitizer from cells to medium was not observed.

Surviving cells were quantified after re-incubation with culture medium with the use of Trypan blue dye exclusion viability assay (Sigma-Aldrich, South Africa) after 48 hrs.

*2.3.3. Phototoxicity test*

The effects of the phantoms were determined by incubating the cells with 10 µg/ml Photosense® for 24 hours. Cells were seeded into 6-well tissue culture plates at a density of 50 cells/mm$^2$ in 3 ml of culture medium. Cells were allowed to attach for 48 hours before being washed twice with 2 ml DPBS, then photosensitized by the addition of culture medium containing 10 µg/ml Photosense®. Control wells contained medium *without* Photosense® and *0 J/cm$^2$* diode laser irradiation (no laser irradiation). Positive control wells contained medium *with* Photosense® and *0 J/cm$^2$* diode laser irradiation. Plates were incubated at 37 °C in 5 % $CO_2$ in the dark for 24 hours, the wells were then washed twice with 2 ml DPBS and the culture medium replaced with 3 ml of DPBS. Wells containing experimental cells (cells treated with Photosense®) were irradiated either with a 676 nm diode laser alone or with the diode laser in the presence of phantoms with a power density of 41 mW/cm$^2$ and irradiation at 4,5 J/cm$^2$ for 14 min (laser alone) or 32 min in the presence of phantoms, respectively. The DPBS was then replaced with 3 ml of fresh supplemented culture medium and the preparation returned to the incubator for further 48 hours. Surviving cells were quantified after re-incubation with culture medium with the use of Trypan blue dye exclusion viability assay after 48 hours.

*2.3.4. Changes in cell viability*

Change in morphology and viability of cells (controls and experimental) was assessed using an inverted microscope. A Trypan blue dye, cell viability assay reagent, based on the principle that live cells possess intact cell membranes that exclude trypan blue was used. The dye exclusion test is used to determine the number of viable cells present in a cell suspension. 50µl trypan blue reagent was added to 50 µl of cell suspension then visually examined to determine whether cells take up or exclude the dye. A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. Cells were counted within 3 to 5 min of mixing cells with trypan blue dye.

## 3. Results

This section is divided into the different sections.

### 3.1. Phantom results

The optical properties of the two different phantom sets were measured on the IS system. The results of the A_1 samples (very low concentration of carbon black), could not be used for the computer model due to the inaccuracies of the results. These values falls outside parameters for the IS model.

The values for the A_3 samples are given in Table 1. All the phantoms had a diameter of 30 mm and a thickness of 1 mm.

Table 1: Optical properties of Phantom A_3 Samples cut into the 1 mm thick discs.

| Phantom name/Property | $\mu_a$ ($mm^{-1}$) (λ=676nm) | $\mu_s$ ($mm^{-1}$) (λ=676nm) |
|---|---|---|
| A_3(I) | 0.15 | 17.89 |
| A_3(II) | 0.12 | 14.86 |
| A_3(III) | 0.12 | 15.35 |

3.2. Computer model predictions

The results in Table 1 were in used in the computer model, simulating a single phantom layer A_3. The fraction of the light that were not absorbed or scattered in the back-ward direction (transmitted light) was compared with the results from the IS measurements and are tabulated in Table 2. In previous studies [6,8] we showed that the comparison between the IS measurement and the computer model predictions is less than 10% at λ=632.8 nm. For this study we used a wavelength of 676 nm (the absorption wavelength of the Photosense®).

Table 2: Comparison between IS and computer model for the light reflected back from the sample (R) and the light transmitted through the sample (T).

| Phantom | R(IS) | T(IS) | R(model) | T(model) | % error in R | % error in T |
|---|---|---|---|---|---|---|
| A_3(I) | 0.39 | 0.29 | 42.79 | 21.37 | 11.04 | 25.36 |
| A_3(II) | 0.38 | 0.33 | 41.88 | 26.12 | 9.05 | 21.65 |
| A_3(III) | 0.39 | 0.33 | 42.19 | 25.45 | 9.14 | 21.78 |

Due to the inaccuracies in the IS model and therefore the prediction values, the actual transmitted values through the phantoms (all 6) were measured and used to calculate the increased treatment time of the cells to ensure the cells with the phantoms and those without the phantoms receive the same total dose during the PDT treatment.

3.3. Cell tests

*3.3.1. Cytotoxicity tests*

Cytotoxicity of Photosense®, as measured by quantifying surviving cells using the trypan blue dye exclusion reagent viability assay 48 hours after the applied treatment, was found to be significant. Cytotoxicity tests showed that at 80 µg/ml Photosense® only 27% of the cells survived, however, with 10 and 20 µg/ml more than 60 % of cells survived (Figure 1). The higher the concentration of Photosense®, the higher is the loss of cell viability of SCC cells.

Upon irradiation with diode laser at 676 nm, cells viability of cells increased in a dose dependant manner, accelerated levels of cell growth were observed with light dose at 5 $J/cm^2$ laser irradiation. The results are in support of previously reported data [9] that laser irradiation at certain fluences and wavelengths can enhance the release of growth factors from cells and stimulate cell proliferation *in vitro*. As another control to prove that the phantoms do not have any adverse effect on the cells, cells were incubated with phantoms for a maximum period of 2 hours to evaluate any loss of cell viability with phantoms and no effect on cell viability was observed.

*3.3.2. Photodynamic effect*

Phototdynamic therapy (PDT) is an established cancer treatment modality that involves the combination of visible light and a photosensitizer. Each factor is harmless by itself, but when combined, in the presence of oxygen, can produce lethal cytotoxic agents that can destroy tumour cells [10]. The combination of 10 µg/ml Photosense® with 4, 5 $J/cm^2$ diode laser at 676 nm, was phototoxic to the SCC cells. Upon irradiation of cells with laser only or in the presence of phantoms, a more

significant decrease in cell viability was observed. Viability studies have shown that phantom do mimic the outer skin layer, hence it takes longer to irradiate cells in the presence of phantoms to achieve phototoxic effect as obtained with laser only.

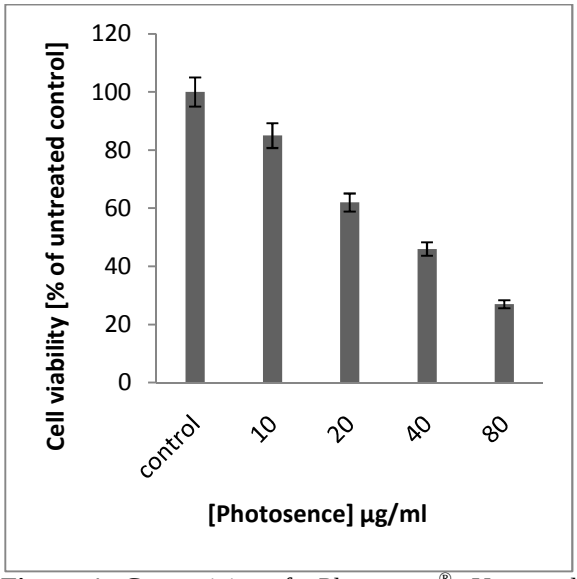


**Figure 1**. Cytotoxicity of Photosense®. Untreated SCC cells (control; 0 µM of Photosense®) were compared with those treated with 10–80 µg/ml of Photosense®.

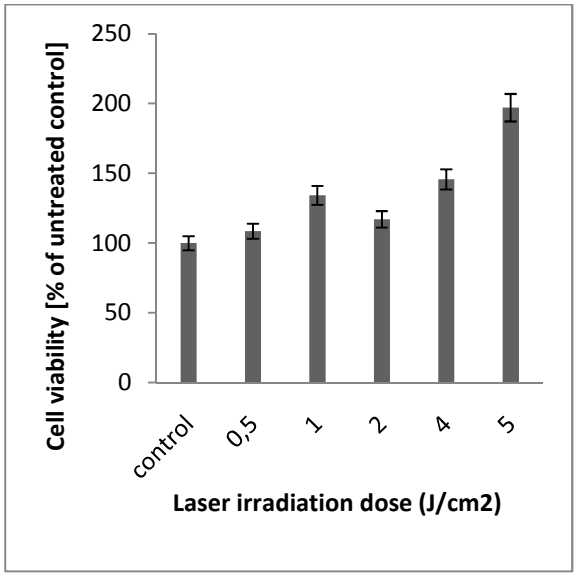


**Figure 2**. Cytotoxicity test of 676 diode laser. Untreated SCC cells (control; 0 J/cm2) of diode laser were compared with those treated with 0, 5 – 5 J/cm$^2$.

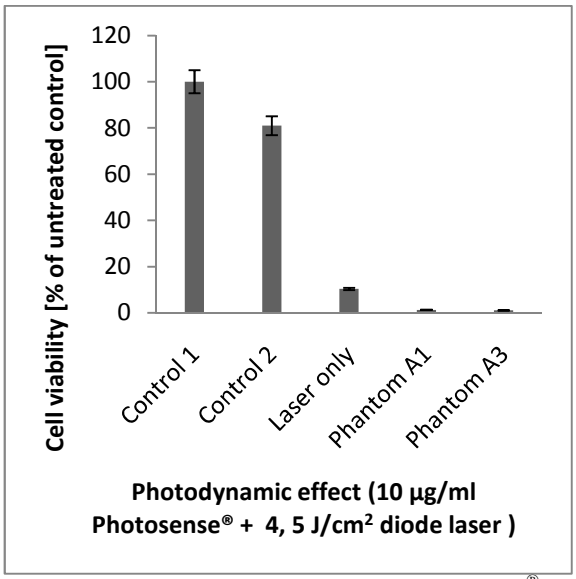


**Figure 3**. Photodynamic effect. Untreated SCC cells (without Photosense® and un-irradiated, Control 1) were compared with those treated with 10 µg/ml of Photosense® and irradiated with 4,5 J/cm$^2$. Further control: SCC cells un-irradiated but treated with 10 µg/ml Photosense® (Control 2).

## 4. Discussion and conclusions

In this work, the suitability of solid phantoms as a replacement for the outer skin layers was investigated. The phantoms were measured with an IS system to obtain the optical properties. These

properties can be used in a computer model to predict the fluence that is transmitted through the model. Even thought the IS model is not as accurate as one would desire, it can already be used for predictions, but needs to be refined to allow for data with very low absorption coefficients.

The cell work proved that the phantoms do not have any adverse effect on the cells in the absence of any treatment parameters. Similar results are obtained when the phantom is placed on the cells to mimic the skin layers and the treatment times are adjusted to allow for the same dose of treatment. This is the first experiment to the authors' knowledge where such a system is used to mimic human skin. The experimental procedures will still have to be refined for more conclusive results.

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