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Low-dose PDT on breast cancer spheroids

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ABSTRACT

Photodynamic therapy (PDT) has been investigated in clinical studies as a treatment method for breast cancer chest wall recurrences. Complete response percentage in these studies is not 100% in most patients, indicating the presence of a remaining tumor after PDT. Some *in vitro* studies show that tumor cells present distinct threshold dose, suggesting that the remaining tumor *in vivo* could require higher doses or different PDT strategies. There is still a lot of controversy of the multiple PDT sessions effect on bulky tumors. The purpose of this study is to investigate low-dose PDT parameters in 3D cultures of breast cancer cells grown by the magnetic levitation method. PDT was performed with Photodithazine® (PDZ) and LED irradiation at 660 nm. Two concentrations of PDZ were investigated and the 50 µg/mL concentration, which showed a superficial distribution, was used in the PDT. Partial damage was observed in the tumors and the viability test showed a small percentage of cell death. This outcome is favorable for the investigation of PDT effects in the remaining tumor. Multiple PDT sections could provide more noticeable alterations in cell morphology and metabolism.

Keywords: Photodynamic Therapy, breast cancer, Photodithazine, magnetic levitation method, 3D culture, MCF-7

1 INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the main cause of death among women worldwide. According to the American Cancer Society, there were an estimated 316,120 new cases of breast cancer expected to be diagnosed in the United States, in 2017.¹ Among the treatment strategies, surgery is performed in most cases and chest wall recurrence after mastectomy (complete removal of the breast) is a frequent problem that lacks of an efficient treatment option. Over the last three decades photodynamic therapy (PDT) has been investigated in clinical studies as a minimally invasive treatment for those recurrent tumors.²⁻⁶ Allison et al. used PDT with Photofrin® in nine patients totalizing 102 sites with lesions diameters from 0.57 to 9 cm. A complete response was observed in 89% of the lesions, the equivalent of 44% of the patients.² Another study using Photofrin® investigated 500 nodules ranging from 1 to 20 mm in fourteen women and 64% presented complete response.⁴ Using Porfimer® as photosensitizer and a protocol of 24-hour illumination, Morrison et al. treated nine patients and one patient (11%) presented complete response and five (56%), partial response.⁶ Liu and colleagues investigated the combination of PDT with a conventional treatment option, the radiotherapy (RT). Two groups of 20 women each were treated with RT alone and with RT plus 5-aminolevulinic acid-PDT. The additive effect of both therapies showed higher complete response (50% versus 20%) and shorter median time to complete response.⁵ In most of these clinical studies, the response is not complete in all patients, evidencing the presence of remaining tumor after PDT. Although PDT is widely known as a therapy that does not induce cell resistance, that might not be entirely true. Clinical and *in vivo* studies have shown that tumor cells became histologically more aggressive after PDT.^{7,8} To better

understand the effects of PDT, including potential resistance, *in vitro* models that represents the complexity of tumors are of great interest.

Three-dimensional (3D) cell culture models are the link between bi-dimensional (2D) cell culture and *in vivo* models. One limitation of monolayer cultures is the restricted cell-cell and cell-extracellular matrix signaling. These signals are related to various cellular processes such as cell differentiation, proliferation, gene expressions, apoptosis and drug response.^{9,10} *In vitro* studies comparing 2D and 3D cultures models show that cells response to a treatment is different for each model. Imamura et al. showed that some breast cancer cell lines presented higher resistance to chemotherapeutical drugs when grown in 3D model compared to 2D cell culture.¹¹ Similar outcomes were observed in a study comparing both models treated with PDT. For the same FS concentration and light exposure, more cells died in the 2D model.¹² Among the 3D models, the magnetic levitation method provides tumors of the order of millimeters, similar to the size found in chest wall recurrences.

In this scenario, the purpose of this study was to investigate low-dose PDT parameters in 3D cell cultures grown in magnetic levitation model. Photosensitizer concentration and light fluence to cause partial damage was determined. These parameters will be used in multiple PDT sections to investigate the possible resistance effects, metabolism and histological changes.

2 MATERIALS AND METHODS

2.1 Cell culture

Human breast adenocarcinoma cell line MCF-7 (ATCC, Manassas, USA) was cultured in phenol red RPMI 1640 (Cultilab, Campinas, Brazil) supplemented with 10% of fetal bovine serum (FBS, Cultilab, Campinas-SP, Brazil) and maintained in an incubator at 37°C and 5% CO₂. For the 3D culture, the 24-well configuration of Bio-Assembler™ System from n3D Biosciences (Houston, USA) was used. Cells grown in 25 cm² flasks were incubated overnight (16 h) with Nanoshuttles™ (NS, n3D Biosciences, Houston, USA) at a concentration of 1,2 µL of NS per 10,000 cells. Cells were trypsinized and 10⁵ cells/well were seeded in 24-well ultralow-attachment plate (Corning, Inc, Tewksbury, USA). The magnetic drive was placed on the top of the plate to induce the magnetically levitated cultures formation.

2.2 Photodithazine dark cytotoxicity

Two PDZ concentrations, 50 and 100 µg/mL, were used to verify the cytotoxicity of the FS in 3 tumors (N=3). Tumors were incubated for 24 h and the plate was covered and kept inside the incubator without the magnetic drive. Wells were washed twice with phosphate buffered saline (PBS) and the MTT (Sigma-Aldrich) solution in fenol red-free media were added and let to react for 4 h. After the addition of DMSO to dissolve the formazan crystals, the absorbance was measured at 570 nm. Tumors without the FS were used as control. Absorbance values were plotted in a graph using the software Origin 9.0 (OriginLab Corporation, Northampton, MA, USA) and they were normalized by the control. Error bars represents standard deviation.

2.3 Photodithazine distribution

Tumors were incubated with PDZ at the concentration of 50 and 100 µg/mL in phenol red-free media. After 24 h, they were washed twice with PBS and transferred to cryomolds filled with O.C.T. compound (Optical Cutting Temperature, Tissue-Tek, Sakura Finetek, USA). Cryomolds were submerged in liquid nitrogen allowing the solidification of the block and they were kept in a -80°C freezer. Frozen samples were sectioned into 50 µm thick slices, placed on microscope slides and imaged on Axio Observer.Z1 fluorescence microscope (Zeiss, Oberkochen, Germany). Samples were excited at 350 ± 30 nm and all the fluorescence above 400 nm was collected.

2.4 Photodynamic therapy

Between the 5th and 9th day of growth, tumors were incubated with PDZ at a concentration of 50 µg/mL. After 24 h, tumors were washed twice with PBS and the media was replaced by phenol red-free RPMI. For

illumination, a Biotable® (IFSC, São Paulo, Brazil), which is a device built with 24 LEDs centered at 660 nm, was used to deliver a fluence of 4 J/cm² at a fluence rate of 30 mW/cm². At the same plate there were the PDT and the control groups (light only). After irradiation, the plate was placed in the incubator protected from light.

2.5 Photodynamic therapy evaluation

To analyze the cell viability, a MTT test was performed 24 h after the treatment in 6 tumors (N=6). The MTT was added to the well, the tumors were mechanically disrupted to mix with the MTT and the plate was left in the incubator for 4 h. Then the absorbance was measured at 570 nm. The tumors were also imaged before and after the PDT using the Axio Observer.Z1 fluorescence microscope (Zeiss, Oberkochen, Germany) on bright field mode.

3 RESULTS AND DISCUSSION

3.1 Photodithazine dark cytotoxicity

The two investigated PDZ concentrations were chosen based on previous studies with the 3D magnetic model. Both concentrations were not cytotoxic in the dark. Figure 1 shows the cell viability after 24 h of incubation. These two FS doses were used in the following distribution assessment.

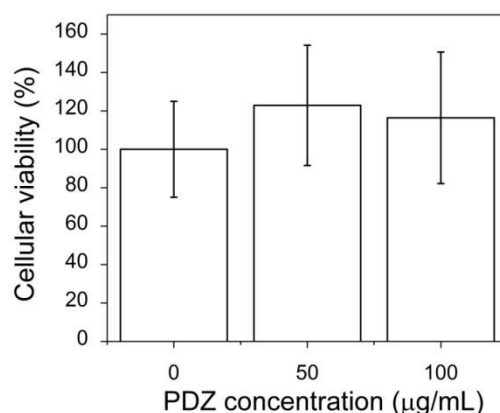


Figure 1. PDZ dark cytotoxicity for 50 and 100 µg/mL. Error bars represents standard deviation. N=3.

3.2 Photodithazine distribution

Tumors grown by the magnetic model have a characteristic of being brown-dark due to the Nanoshuttles. This aspect brings the challenge of imaging the entire tumor by optical techniques such as confocal and two-photon microscopy. To overcome this issue, the cryosection method was used and the slices were imaged by fluorescence microscopy. Figure 2 shows the PDZ distribution for each concentration and for the control. From top to bottom, sections from one edge of the tumor to the other are shown. These cuts evidenced the elongated shape of the tumor spheroids in the x and y dimension compared with the z dimension. The first column shows the sections for the control group (figure 2-a). The green color is the native fluorescence of the cells. When the PDZ is present at the concentration of 50 µg/mL, we can see the red fluorescence mainly on the surface of the tumor (figure 2-b). Superposition of autofluorescence and PDZ fluorescence appears in yellow. At the concentration of 100 µg/mL, the distribution of PDZ was homogeneous in the entire tumor (figure 2-c). Once both concentrations were not cytotoxic, both could be used for PDT. However, for the investigation of PDT effects in a remaining tumor, a partial damage was necessary. Then, we chose the lower concentration to perform PDT.

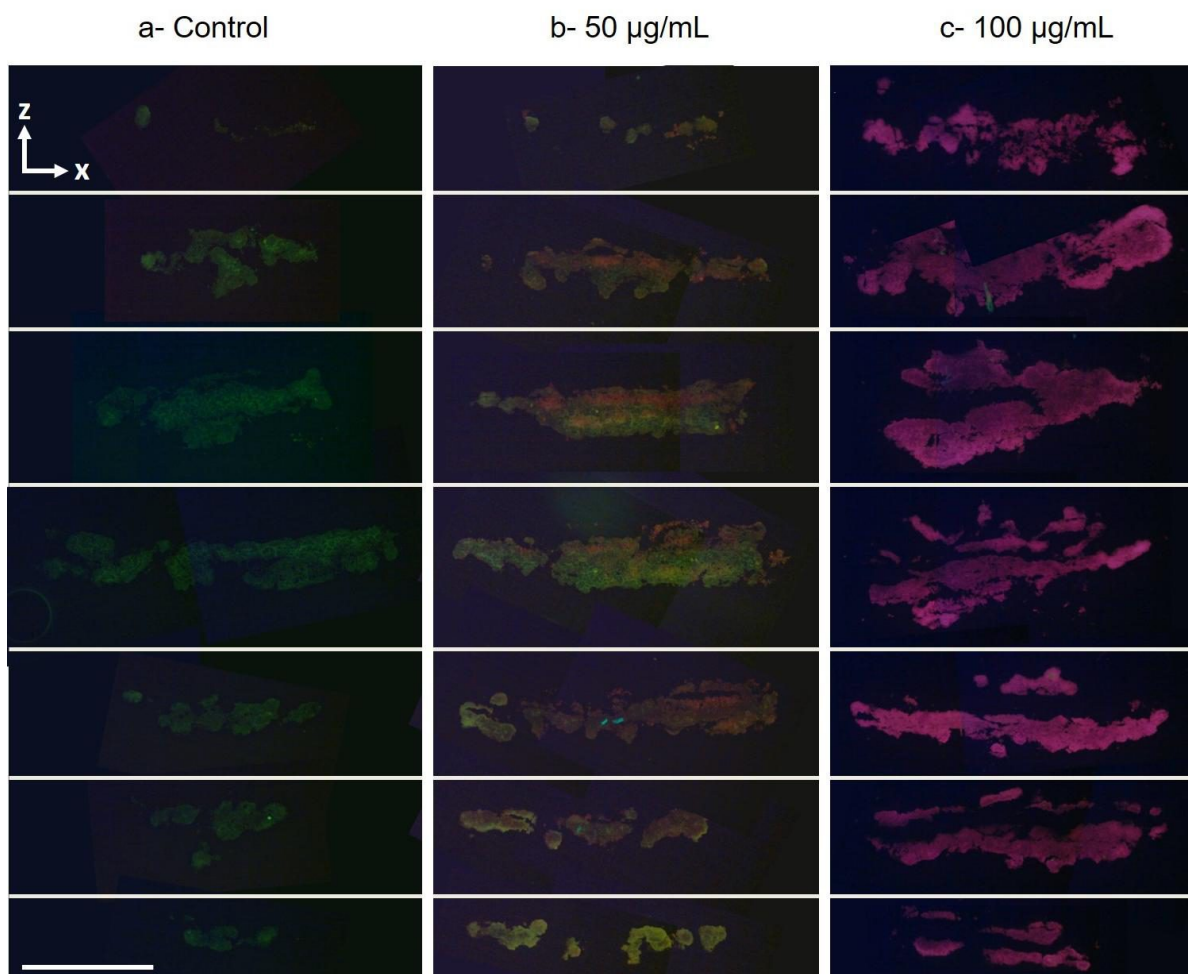


Figure 2. Fluorescence images of tumors cryosections. Column a: control, column b: tumor incubated with 50 $\mu\text{g/mL}$ and column c: tumor incubated with 100 $\mu\text{g/mL}$. From top to bottom, seven sections from one edge of the tumor to the other are show. Sections were 50 μm thick. Green color is the tumor native fluorescence, red is the PDZ fluorescence and yellow is the superposition of green and red. Scale bar: 1 mm.

3.2 Photodynamic therapy evaluation

The macroscopic effects of PDT were assessed by images of the tumors after the illumination. Figure 3 shows the images of 3 tumors from treated (figure 3-a) and 3 from control (figure 3-b) group. Tumors after PDT presented damages such as fragmentation, cohesion loss and holes (figure 3-a) while the control presented only a slight disturbance due to the absence of the magnetic drive. Loss of cohesion was observed by the less dense tumor combined with the increased area of the tumor (figure 3-a, second tumor). This observation is important since the increased area in this case cannot be associated with tumor growth, but the decrease in its density. The viability test corroborated the partial damage observed in the tumor morphology showing a small percentage of cell death (figure 4). This outcome was expected since the FS concentration and low light fluence were chosen with the objective of producing minimal damage.

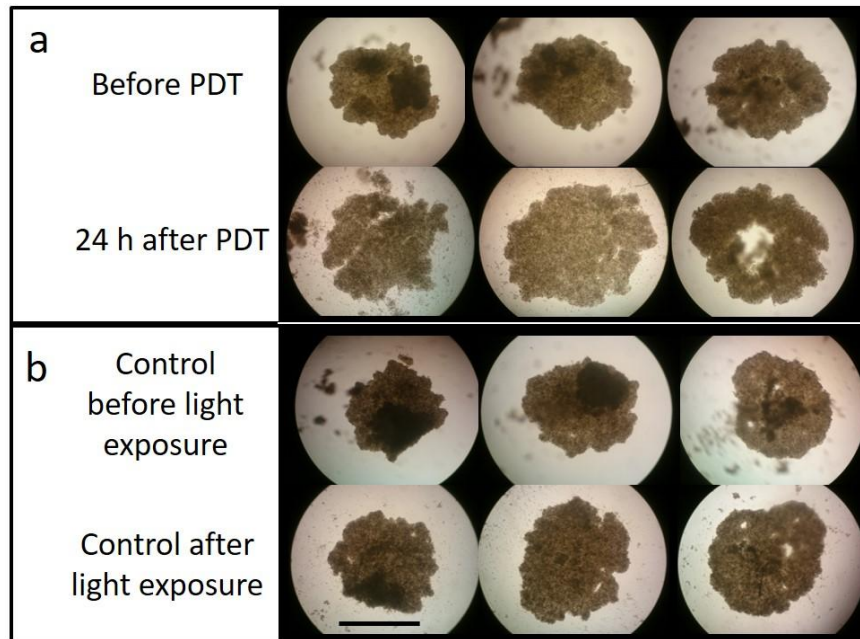


Figure 3. Bright field images of tumors after PDT. A: tumors before and after PDT; b: tumors before and after light exposition (control). Scale bar: 2 mm.

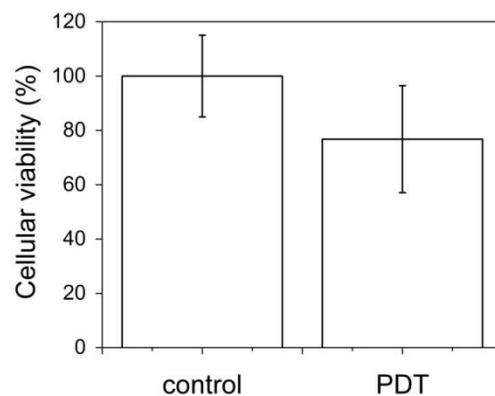


Figure 4. Cellular viability after PDT. Error bars represents standard deviation. N=6.

4 CONCLUSIONS

This work presented the initial investigation of PDT parameter aiming the study of PDT effects in remaining tumors. Photodithazine® concentration of 50 µg/mL and light fluence of 4 J/cm² were the PDT parameters which caused partial damage in the 3D cell cultures of breast cancer. 3D magnetic levitation method provided tumors of the order of milliliters, and then PDT could be investigated *in vitro* with tumors of similar size as *in vivo*. After PDT, tumors presented fragmentation, cohesion loss and holes and these alterations were accompanied by a small percentage of cell death. Therefore, this outcome will contribute to the investigation of PDT effects in 3D cell cultures concerning cell resistance and morphological and metabolic changes.

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REFERENCES

- [1] American Cancer Society., "Breast Cancer Facts & Figures 2017-2018," Am. Cancer Soc. Inc (2017).
- [2] Allison, R., Mang, T., Hewson, G., Snider, W. and Dougherty, D., "Photodynamic therapy for chest wall progression from breast carcinoma is an underutilized treatment modality," *Cancer* **91**(1), 1–8 (2001).
- [3] Allison, R. R., Sibata, C., Mang, T. S., Bagnato, V. S., Downie, G. H., Hu, X. H. and Cuenca, R., "Photodynamic therapy for chest wall recurrence from breast cancer," *Photodiagnosis Photodyn. Ther.* **1**(2), 157–171 (2004).
- [4] Cuenca, R. E., Allison, R. R., Sibata, C. and Downie, G. H., "Breast cancer with chest wall progression: treatment with photodynamic therapy.," *Ann. Surg. Oncol.* **11**(3), 322–327 (2004).
- [5] Liu, Y., Hou, G., Zhang, X., Liu, J. J., Zhang, S. and Zhang, J., "A pilot randomized clinical study of the additive treatment effect of photodynamic therapy in breast cancer patients with chest wall recurrence," *J. Breast Cancer* **17**(2), 161–166 (2014).
- [6] Morrison, S. A., Hill, S. L., Rogers, G. S. and Graham, R. A., "Efficacy and safety of continuous low-irradiance photodynamic therapy in the treatment of chest wall progression of breast cancer," *J. Surg. Res.* **192**(2), 235–241 (2014).
- [7] Fiechter, S., Skaria, A., Nievergelt, H., Anex, R., Borradori, L. and Parmentier, L., "Facial basal cell carcinomas recurring after photodynamic therapy: A retrospective analysis of histological subtypes," *Dermatology* **224**(4), 346–351 (2012).
- [8] Gilaberte, Y., Milla, L., Salazar, N., Vera-Alvarez, J., Kourani, O., Damian, A., Rivarola, V., Roca, M. J., Espada, J., González, S. and Juarraz, A., "Cellular intrinsic factors involved in the resistance of squamous cell carcinoma to photodynamic therapy," *J. Invest. Dermatol.* **134**(9), 2428–2437 (2014).
- [9] Kim, J. Bin., "Three-dimensional tissue culture models in cancer biology.," *Semin. Cancer Biol.* **15**(5), 365–377 (2005).
- [10] Xu, X., Farach-Carson, M. C. and Jia, X., "Three-dimensional in vitro tumor models for cancer research and drug evaluation," *Biotechnol Adv* **32**, 1256–1268 (2014).
- [11] Imamura, Y., Mukohara, T., Shimono, Y., Funakoshi, Y., Chayahara, N., Toyoda, M., Kiyota, N., Takao, S., Kono, S., Nakatsura, T. and Minami, H., "Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer," *Oncol. Rep.* **33**(4), 1837–1843 (2015).
- [12] Chen, Y.-C., Lou, X., Zhang, Z., Ingram, P. and Yoon, E., "High-Throughput Cancer Cell Sphere Formation for Characterizing the Efficacy of Photo Dynamic Therapy in 3D Cell Cultures.," *Sci. Rep.* **5**, 12175 (2015).