

Getting it right – 3D Cell cultures for the assessment of photosensitizers for photodynamic therapy (PDT)

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Core quote “Cancer drug screening is rapidly moving towards the use of 3D cell models. The added complexity of PDT action makes this a *conditio sine qua non* for the screening of new photosensitizers.”

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The quest for the treatment of malignant diseases is ongoing. Classic modalities based on chemo- and radiotherapy and surgery are now supplanted by or used in conjunction with photodynamic therapy (PDT) [1]. PDT involves on the administration of a photosensitizing dye and its accumulation in the affected tissue. After illumination of the respective body part with light (typically low intensity lasers), the photosensitizer transfers its excitation energy to triplet oxygen (the ‘regular’ form of oxygen in the body) to yield singlet oxygen and other reactive oxygen species (ROS). ROS are highly reactive and cytotoxic, resulting in cell death *via* apoptosis, necrosis, or autophagy [2]. PDT can be very effective for treating cancers and has found widespread use in dermatology - *e.g.*, as PUVA treatment for actinic keratosis - and is now approved for treating cancer of the esophagus, non-small cell lung cancer and certain types of head & neck cancers or dysplasias. Prime examples for photosensitizers are porphyrins, chlorophyll derivatives, δ -aminolevulinic acid (precursor for protoporphyrin), simple dyes such as psoralen, and others [3].

Yet, despite its promise, widespread clinical use of PDT is only slowly emerging; in part the result of drawbacks of existing drugs and the slow entry of new drugs into the developmental pipeline. Photosensitizers are often classified as 1st, 2nd and 3rd generation drugs, with Photofrin being the classic 1st generation PDT drug. This was followed by improvements of the photophysical and pharmacological properties in 2nd generation molecules such as Levulan, Temoporfin or Verteporfin [2]. Currently, a significant number of similar drugs are in preclinical development. Contemporary studies on 3rd generation agents focus on improved targeting, either *via* bioconjugate strategies (*e.g.*, carbohydrate or antibody appended porphyrins), as nanoformulations or active transport systems [3,4].

An optimum photosensitizer should have a high singlet oxygen quantum yield, not undergo photobleaching, be non-toxic and stable in the dark, water soluble, and be

selectively retained in high concentrations in the target tissue and readily clear from the body in order to avoid continued photosensitivity. Through advances in the synthetic chemistry of porphyrins and related compounds, chemists can design and synthesize 'optimized' photosensitizers with a high level of sophistication [3]. However, this is no guarantee for their *in vitro* and *in vivo* efficacy. Parameters such as tissues clearance, photosensitivity, (dark) phototoxicity, pain and relative enrichment in target tissue are difficult to predict and can only be assessed through cell biological studies. Typically, new photosensitizing agents are evaluated *in vitro* by investigating the cell viability in simple "2D" cell cultures and comparing the results from dark and illuminated samples.

As for any other drug development such *in vitro* cytotoxicity tests can only give an indication for the *in vivo* pharmacology and the ultimate utility of a lead compound. The different levels of complexity still results in many of the *in vitro* leads failing at the *in vivo* level, with only 5% of anti-cancer drugs making it from *in vitro* studies to the clinic, illustrating how the success of a compound *in vitro* does not necessarily translate to *in vivo* studies [5]. In terms of cancer treatment several critical aspects of PDT cannot be modeled at all in standard *in vitro* cell test. In addition to direct cell killing, a significant part of the "PDT effect" are tissue related, *e.g.*, damage of the microvasculature *via* anti-angiogenesis and anti-tumor immune responses. Additionally, PDT relies on the availability of oxygen in the target tissue and thus tumor oxygenation and hypoxia impact the overall treatment effect [2].

Clearly, standard 2D monolayer cell cultures do not accurately recreate the tumor environment. Next to obvious features such as the absence of blood vessels and different oxygenation states, cell motility in tissue, many other issues limit the predictive utility of 2D cell cultures. Cells growing in monolayer are more susceptible to toxins and physiologically constrained. Likewise, cells growing in a monolayer on a flat adherent substrate have drastically reduced cell-cell and cell-extracellular matrix. Herein lays the motivation to expand into the third dimension – the ability of a cell culture to accurately model molecular gradients that modulate the transport of soluble components such as oxygen and signaling molecules within the cell mass, together with aspects of the tissue organization and structure, are critical aspects of the diseased tissue and must be taken into account early on in the drug testing [6]. The native tumor environment can only be accurately recreated – or at least modeled – in 3D cell cultures [7].

Studies have shown that candidate drugs are significantly less effective in 3D compared to similar studies conducted using 3D cell cultures, a clear indication of the significant effect that the tumor microenvironment has on the response to treatment. For example, the sensitivity of OVCAR5 cells in 3D cell cultures is half of that observed in a 2D monolayer environment [8]. 3D systems more closely represents the *in vivo* response not only in terms of architecture and nutrient supply, but also with regard to the expression of disease biomarkers and the activity of signaling pathways [nature]. The different chemosensitivity in 2D *versus* 3D systems can be attributed to a number of features that arise from the tumor architecture, including the penetration of the drug through multiple layers of cells and the activation of signaling pathways which are suppressed in 2D cell cultures [5]. In 3D cell cultures, the PS cannot normally easily reach the inner most tumor cells; there is also an overexpression of hypoxia genes which depletes the oxygen concentration in the cell. This effect is even more exacerbated in PDT due to oxygen consumption [9].

The last years have seen the emergence of several different types of 3D cell cultures, for example tissue explants and cellular spheroids [6,7,10]. Especially spheroid structures are used more and more; however, a major limitation is the difficulty in scaling them up uniformly for drug screening. Additionally, they exhibit slow spontaneous aggregation and one lacks control over their final size and shape. Likewise, the ability to study physiological signals is also limited due to the diffusion gradients in gel based systems. Nevertheless, spheroid cultures have served their purpose in opening the door to major conceptual advances [10].

These obstacles can be overcome by using self-assembling hydrogels as molecular scaffolds [9]. It allows control of size and shape, and thus tunes the molecular gradient and the oxygen concentration in the tumor model. In principle one can now alter the 3D model to fit a specific type of cancer. For example, ovarian cancer results from the attachment of single cells onto a peritoneal surface, which eventually leads to micro nodular tumors. This can be modelled by seeding cells on a Matrigel basement membrane forming 3D acini. The physical characteristics observed for this model were found to be highly varied, and thus representative of the actual tumor microenvironment – structures ranged from small clusters to large acini to caked accumulations of multiple acini, all of which resembles the heterogeneity of the disease in the body [8]. Additionally, the ‘entrapment’ of cells in the matrix gives more accurate data on cell death [11].

Still, this advancement has its limitations, too. For PDT drugs these relate to issues with imaging and how to monitor the overall efficacy of PDT in 3D *in vitro* models. Thus, development of high content screening methods can be more difficult for 3D models, but reliable protocols have emerged [8,12]. At a more fundamental level the question arises whether different photochemical mechanisms are present in 2D or 3D *in vitro* and *in vivo* systems and how this can be utilized for the *de novo* design of photosensitizers [9,13]. Analytical problems relate to the limited ability of microscopes for visualizing complex spheroid structures requiring special quantitative imaging techniques [14]. Other PDT specific issues of 3D cultures relate to the light component of the treatment [13]. Dosimetry can be problematic, typically analysis centers on measurement of the fluence rate, which does not take the optical properties or geometry of the tumor into account [15]. Additionally, problems arise from the use of different imaging methods in different studies, which makes a comparative analysis of drug candidates from different laboratories difficult.

Nevertheless, studies from recent years indicate the validity of the “3D approach” for photosensitizer testing. These include the use of self-assembling peptides as a cellular scaffold to model drug and oxygen diffusion effects indicating that mass transfer is the main factor differentiating 2D and 3D responses [9]. 3D cultures can be used to evaluate the theranostics potential of new drugs [16]. Other recent examples are the modeling of tumor-stroma interactions [17], mixed neuron/cancer cell systems [11], the specific optimization of PDT drugs for hypoxic tumor environments [12], two-photon PDT studies [18], and a microfluidal breast cancer tissue model based on lab-on-a-chip technology [19], to name a few. Another noteworthy development is the use of optical coherence tomography for the visualization and study of 3D PDT models [20], which indicates the analytical and predictive power of contemporary imaging techniques in conjunction with modern tissue models.

Clearly, the realm of *in vitro* testing for PDT is rapidly moving into the third dimension. While all cancer drug development appears to slowly moving towards the use of 3D cell models the added complexity of PDT action makes this a *conditio sine qua non* for the screening of new photosensitizers.

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References

- 1 Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q. Photodynamic therapy. *J. Natl. Cancer Inst.* 90, 889–905 (1998).
- 2 Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one- photosensitizers, photochemistry and cellular localization. *Photodiagn. Photodyn. Ther.* 1, 279–293 (2004).
- 3 Ethirajan M, Chen Y, Joshi P, Pandey RK. The role of porphyrin chemistry in tumor imaging and photodynamic therapy. *Chem. Soc. Rev.* 40 340–362 (2011).
- 4 Rogers L, Senge MO. The translocator protein as a potential molecular target for improved treatment efficacy in photodynamic therapy. *Future Med. Chem.* 6, 775–792 (2014).
- 5 Lee JM, Mhawech-Fauceglia P, Lee N, Parsanian LC, Lin YG, Gayther SA, Lawrenson K. A three-dimensional microenvironment alters protein expression and chemosensitivity of epithelial ovarian cancer cells *in vitro*. *Lab. Invest.* 93, 528–542 (2013).
- 6 Pampaloni F, Reynaud EG, Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. *Nature Rev. Mol. Cell Biol.* 8, 839–845 (2007).
- 7 Baker BM, Chen CS. Deconstructing the third dimension - how 3D culture microenvironments alter cellular cues. *J. Cell Sci.* 125, 3015–3024 (2012).
- 8 Rizvi I, Celli JP, Evans CL, Abu-Yousif AO, Muzikansky A, Pogue BW, Finkelstein D, Hasan T. Synergistic enhancement of carboplatin efficacy with photodynamic therapy in a three-dimensional model for micrometastatic ovarian cancer. *Cancer Res.* 70, 9319–9328 (2010).
- 9 Alemany-Ribes M, García-Díaz M, Busom M, Nonell S, Semino CE. Toward a 3D cellular model for studying *in vitro* the outcome of photodynamic treatments: Accounting for the effects of tissue complexity. *Tissue Eng. Pt. A.* 19, 1665–1674 (2013).
- 10 Breslin S, O’Driscoll L. Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov. Today* 18, 240–249 (2013).
- 11 Wright KE, Liniker E, Loizidou M, Moore C, MacRobert AJ, Phillips JB. Peripheral neural cell sensitivity to mTHPC-mediated photodynamic therapy in a 3D *in vitro* model. *Br. J. Cancer.* 101, 658–665 (2009).
- 12 Klein OJ, Bhayana B, Park YJ, Evans CL. *In vitro* optimization of EtNBS-PDT against hypoxic tumor environments with a tiered, high-content, 3D model optical screening platform. *Mol. Pharmaceutics* 9, 3171–3182 (2012).
- 13 Salas-García I, Fanjul-Vélez F, Arce-Diego JL. Superficial radially resolved fluorescence and 3D photochemical time-dependent model for photodynamic therapy. *Opt. Lett.* 39, 1845–1848 (2014).
- 14 Celli CP, Rizvi I, Evans CL, Abu-Yousif AOA, Hasan T. Quantitative imaging reveals heterogeneous growth dynamics and treatment-dependent residual tumor distributions in a three-dimensional ovarian cancer model. *J. Biomed. Opt.* 15, 051603 (2010).
- 15 Rizvi I, Anbil S, Alagic N, Celli JP, Zheng LZ, Palanisami A, Glidden MD, Pogue BW, Hasan T. PDT Dose Parameters Impact Tumoricidal Durability and Cell Death Pathways in a 3D Ovarian Cancer Model. *Photochem. Photobiol.* 89, 942–952 (2013).

- 16 Glidden MD, Celli JP, Massodi I, Rizvi I, Pogue BW, Hasan T. Image-Based Quantification of Benzoporphyrin Derivative Uptake, Localization, and Photobleaching in 3D Tumor Models, for Optimization of PDT Parameters. *Theranostics* 2, 827–839 (2012).
- 17 Celli JP. Stromal interactions as regulators of tumor growth and therapeutic response: A potential target for photodynamic therapy? *Isr. J. Chem.* 52, 757–766 (2012).
- 18 Liu J, Li G, Zhang P, Jin C, Zeng L, Ji L, Chao H. Ruthenium(II) polypyridyl complexes as mitochondria-targeted two-photon photodynamic anticancer agents. *Biomaterials* 56, 140–153 (2015).
- 19 Yang Y, Yang X, Zou J, Jia C, Hu Y, Du H, Wang H. Evaluation of photodynamic therapy efficiency using an in vitro three-dimensional microfluidic breast cancer tissue model. *Lab. Chip.* 15, 725–744 (2015).
- 20 Jung Y, Nichols AJ, Klein OJ, Roussakis E, Evans CL. Label-Free, Longitudinal Visualization of PDT Response *In Vitro* with Optical Coherence Tomography. *Isr. J. Chem.* 52, 728–744 (2012).

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