**Abstract:** A pointsource photodynamic therapy (PDT) technique has been developed to deliver sensitizer, light and oxygen that are precursors to singlet oxygen (a cytotoxic excited state of O₂) in a highly localized and controllable fashion. The characterization of cell killing radius in vitro provides insight into the release kinetics and diffusion of the sensitizer. This opens the door to an instrument that brings all components for PDT to a device tip and may provide long-sought improvements in blood-rich or turbid media and in dosimetry.

**Keywords:** rectilinear photodynamic therapy; teflon tips; singlet oxygen halo; sensitizer drift.

1 **Objective**

The discovery of high-precision techniques to eradicate tumors would be of tremendous use for surgeons. Removal of tumors that are directly adjacent to vital tissue is a daunting challenge. Radiation and chemotherapy have been in the limelight for years in non-surgical treatments, but the field needs further advances in high-precision treatment delivery.

2 **Method**

One solution to this problem is a pointsource photodynamic therapy (PSPDT) device that channels O₂ and red diode laser light through a probe tip to the tumor site (Figure 1) [1]. The probe tip discharges sensitizer molecules that serve as precursors to singlet oxygen (¹O₂), a key cytotoxic species, placing all components necessary for PDT at the tumor and nowhere else in the body.

3 **Results and discussion**

Our device has demonstrated photokilling activity and precision in glioma U-87 cells in vitro by creating a halo of singlet oxygen (Figure 2) [2]. The photokilling was analyzed by a live/dead assay, with a circle of killing around the probe tip. The probe tip design seemed reasonable. When placed 0.25 mm above U-87 cells spread into a monolayer on a microwell plate, the cell killing radios proceeded from 0.1 to 2.9 mm with increased treatment times.

There is intuition and rationale in the probe tip design. The thinking was that a means for sensitizer release to allow for its excitation while in the tissue was needed due to the short diffusion distance of ¹O₂ delivered extracellularly to tissue. Once in use, the device kills cancer cells in regions where the sensitizer diffuses, i.e. beyond the tip’s point of application. This combination of sensitizer release and ¹O₂ generation provides a resolution for the ¹O₂ paradox. Nature
has provided us laws that govern singlet oxygen’s existence, namely its short lifetime and diffusion distance. The released sensitizer effectively lengthens singlet oxygen’s diffusion distance in the guise of photocompanion catalysis (synchronizing sensitizer location to \(1O_2\) generation). Figure 2 shows there is no requirement for the tip to touch each margin in order to kill cells; notice the killing occurred beyond the dashed white line where the probehead actually touched. Furthermore, these doses do not result in increased heat, and the amount of time required to point the device tip at each cell margin is tunable, as is seen next.

Data from U-87 and OVCAR-5 cells suggested that nonlinear sensitizer release depends on fluorination of the tip [2, 3], which led to curved Eyring plots of \(\ln k\) (autocatalytic fitting) vs \(1/T\). A mechanism was proposed for the sigmoidal photorelease process [4]. In Figure 3 shown in green, with the native silica, there is sensitizer photorelease with non-sigmoidal kinetics. Shown in red, with the fluorinated silica, the rapid photorelease is forestalled until higher concentrations of released sensitizer become available. Shown in blue, immediate acceleration is the result when cleaved sensitizer is added as a dopant at \(t=0\) min. When cleaved sensitizer is spiked into solution, the induction step is eliminated. That the induction period can be bypassed is quite informative. The steepest sigmoid was seen at 20 °C. As the temperature \(T\) increased from 20 to 100 °C, \(k\) decreased by 30%. This can be understood in terms of an entropy-controlled reaction with lower product formation at higher temperature from lower reactivity of singlet oxygen with the ethene, and the negative activation energy rises by a tenth of a kJ/mol.

Design and synthesis of sensitizer compounds affect the delivery system characteristics. The extent of sensitizer PEGylation [5], for example, can regulate the time required for the tumor cells to uptake the sensitizer prior to light and \(O_2\) delivery to increase applicability in a clinical setting. Sensitizers bind into membranes at different rates based on PEGylation and other factors. Sensitizer in-diffusion arrival provides the relative position of \(1O_2\) that will form. Thus far, we know the phototoxic impact of our device tip can be tuned by sensitizer type and conditioning, such as fluorination.

Probe tip fluorination is found to improve repellent and other properties [6]. In addition to non-fluorinated and fluorinated glass tips, we have also examined polyvinyl alcohol (PVA) and teflon/PVA nanocomposites [7]. Fluorinated media lead to a reduction in the adsorbive
affinity of the departing sensitizer with improved release to cellular or liquid surroundings. The advantages of the fluorinated over non-fluorinated surface go beyond the self-cleaning properties, there is a small O2 solubility enhancement caused by the fluorinated surface and also an enhanced percent cleaving efficiency. The fluorinated surfaces also protect 1O2 from strong surface physical quenching compared to native silica or PVA allowing it to escape beyond the probe tip. The replacement of the O–H groups for the C–H and C–F groups enhanced the 1O2 lifetime at the tip interface due to less efficient electronic-to-vibronic energy transfer and 1O2 quenching.

In turning out sensitizer from the tip, control is desired, a reasonable platform for continuous or paced delivery is needed for good dosing. Flooded, acute or abrupt turnout leads to aggregation of the sensitizer and low 1O2 photo-production efficiency. Aggregated sensitizers are interlopers in a sense; e.g., they hinder good photophysics by causing shorter excited-state lifetimes. A counterpart, a probe tip that flows 'O2 too rapidly (like throwing fuel on a fire) would also not be the way to go. Thus, our fluorinated probe tips can be suited to pace sensitizer release. Our data show that the fluorinated silica surface becomes privy to autocatalytic-assisted release kinetics, which has significant potential to direct local phototoxicity via singlet oxygen. Autocatalysis (or even oscillating kinetics) provides a mix of good advantages in the operation of the instrument. Control of spatial distance between the sensitizer molecules is also desired so as not to fret when >15 Å distances exist for maximal photocleavage; otherwise, self-quenching by neighboring sensitizer molecules occurs by FRET (pun intended). Optimal sensitizer photorelease has been examined for the probe tip, where crowding of the sensitizer molecules and self-quenching were kept to a minimum.

4 Conclusion

We feel the time is ripe for pointsource PDT to be tested in an intraoperative setting in treating residual disease. What needs to be scrutinized is a look beyond pilot feasibility data to a tumor model to see if the technique will be useful in vivo. Treatment of head and neck cancers seem promising. With proper controls, future studies can test this device and that if it will work efficiently or better that any currently available treatment. Questions about the device tip’s efficacy in assorted geometric shapes or by silicone deposition or three-dimensional printing for rectilinear singlet oxygen delivery also need to be answered.

Acknowledgments: AAG, DB, GG, NW, IA and AG acknowledge support from the National Science Foundation (Grant number: ‘CHE-1464975’) and the NIH (Grant number: ‘SC1GM093830’). KAC, JMM and TMB acknowledge support from the NIH (Grant number: ‘P01 CA087971’). We also thank Leda Lee for the graphic arts work.

References


[7.04] Antitumor effect of sinoporphyrin sodium loaded graphene oxide on human liver cancer HepG2 cells

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Abstract: Photofrin-based photosensitizer is the first U.S. Food and Drug Administration (FDA)-approved photosensitizer for clinical application. However, the long-term skin toxicity and low tumor-selectivity limit the clinical application. Various nanocarriers have been developed for the delivery of photosensitizers to improve the poor aqueous solubility and tumor accumulation of photosensitizers. In this study, sinoporphyrin sodium loaded PEGylated graphene oxide (GO-PEG-DVDMS) was prepared to investigate the antitumor effect on human liver cancer cells. HepG2 cells with GO-PEG-DVDMS showed obviously lower cell viability under laser irradiation compared to control experiments without laser irradiation, suggesting the great potential of GO-PEG-DVDMS for liver cancer therapy.

Keywords: graphene oxide; sinoporphyrin sodium; PTT; PDT; liver cancer.

1 Introduction

Phototherapy, represented by photodynamic therapy (PDT) and photothermal therapy (PTT) have aroused wide interest in anticancer therapy due to unique advantages such as remote controllability, improved selectivity, and low systemic toxicity. PDT is an U.S. Food and Drug Administration (FDA)-approved treatment modality, in which a systemically or locally administered photosensitizer is activated locally by irradiating the lesion site with light of a suitable wavelength and power [1]. Furthermore, it has been proved that PDT is an effective treatment method in several cancers. But the long-term skin toxicity and low tumor-selectivity and photobleaching have limited the further clinical application [2, 3]. PTT is a non-invasive therapeutic technique which has unique advantages such as remote controllability, improved selectivity, and low systemic toxicity. Among various nanomaterials, graphene oxide (GO) has been intensely studied by different groups and functionalized GO, which shows strong near-infrared (NIR) absorbance and high drug-loading capacities, for effective photothermal ablation of tumors as well as cancer combination therapy in animal experiments. More recently, GO has also been widely explored as promising drug delivery systems for improved cancer treatment.

Hepatocellular carcinoma (HCC) is a common disease worldwide. The prognosis of HCC is generally poor. Partial hepatectomy remains the best hope for a cure but is suitable for only 9–27% of patients. The presence of significant background cirrhosis often precludes liver resection in patients with HCC [4]. Herein, we loaded an active compound from Photofrin II, named as sinoporphyrin sodium (DVDMS), onto the PEGylated GO with large specific surface area for the non-invasive therapy of HCC [5]. The new synergistic PTT/PDT platform based on the DVDMS-loaded PEGylated GO is an effective approach on cell proliferation inhibition of liver cancer cells.

2 Materials and methods

2.1 Chemicals and reagents

Graphite powder was purchased from Aladdin Reagent Co., Ltd.; mPEG-NH2 (Mw 3500) was purchased from Sigma. Ethylenediamine (EDA), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), n-hydroxysuccinimide (NHS) and indocyanine green (ICG) were obtained from J&K Company (Beijing, China), MTT assay kit and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Bioengineering Co., Ltd. (Shanghai, China). Other reagents were purchased from China National Medicine Corporation and used as received. All solutions were freshly prepared using ultrapure water from a Millipore Milli-Q® system.