A Fiberoptic (Photodynamic Therapy Type) Device with a Photosensitizer and Singlet Oxygen Delivery Probe Tip for Ovarian Cancer Cell Killing

Dorota Bartusik¹, David Aebisher¹, Ashwini Ghogare³, Goutam Ghosh¹, Inna Abramova¹, Tayyaba Hasan² and Alexander Greer¹

¹Department of Chemistry, Graduate Center, City University of New York Brooklyn College, Brooklyn, NY
²Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA

Received 12 February 2013, accepted 8 March 2013, DOI: 10.1111/php.12072

ABSTRACT

A portable “fiber optic-based sensitizer delivery” (FOSD) device has been developed and studied. Before there might be success in photodynamic therapy (PDT) and antibacterial ambitions, an understanding of basic factors on device performance was needed. Thus, the device was examined for the localized delivery of sensitizer molecules in ovarian cancer cells and production of high concentrations of singlet oxygen for their eradication in vitro. The device tip releases stored pheophorbide by attack of singlet oxygen from sensitized oxygen gas delivered through the hollow fiber using 669 nm laser light. The performance of the device was enhanced when configured with a fluorosilane tip by virtue of its Teflon-like property compared with a conventional glass tip (greater sensitizer quantities were photoreleased and laterally diffused, and greater amounts of ovarian OVCAR-5 cancer cells were killed). No cell damage was observed at 2.2 N of force applied by the probe tip itself, an amount used for many of the experiments described here.

INTRODUCTION

We recently reported on a sensitizer-immobilized fiber-optic device as a unique way to deactivate bacteria and photodestroy compounds in water (1,2). More recently, we developed an improved device in which photosensitization was used to release the photosensitizer from the device (3,4).

Figure 1 shows the conceptual cartoon of the “fiber optic-based sensitizer delivery” (FOSD) device and action. The device comes with a porous Vycor glass (PVG) tip that is mounted onto the terminus of the optical fiber.

Probe tip 1 is a pheophorbide photosensitizer covalently bound to PVG (Fig. 1) (3). It is a sensitizer-capped device with a photolabile di-O-vinyl ether bridge that releases the pheophorbide stored on the cap via attack of singlet oxygen from sensitization of the oxygen gas delivered through the hollow fiber using 669 nm laser light. O-Vinyl ethers are useful for photocleaving reactions at the double bond, a number of them react with O₂ by mechanisms involving dioxetane intermediates (5–8). Probe tip 1 has since been modified to probe tip 2 to improve the release action of the pheophorbide sensitizer (9).

Probe tip 2 is a “second-generation” photosensitizer system (Fig. 1) (9). In addition to the photocleavable sensitizer, it contains a surface bound nonafluorosilane. The result is decreased absorptive affinity of the sensitizer on the probe tip surface. However, advantages of probe tip 2 over 1 go beyond the self-cleaning properties, the high number of surface fluorine atoms also produced some quenching properties that were advantageous, including reduced physical quenching of singlet oxygen by the probe interface and enhanced chemical quenching of the ethene site bonding the sensitizer to the probe tip. A formyl ester fragment remains on the surface after photodestruction of the di-O-vinyl ether bridge. Over time, the freed formate ester pheophorbide 3 forms the hydrolyzed benzyl ester pheophorbide 4 and parent pheophorbide-a 5, a process that occurs more rapidly with base than acid (Fig. 2) (3).

We now report on our investigation into the application of the FOSD device for ovarian cancer cell eradication. Although a high number of studies have been conducted with fiber optics as light guides for hospital photodynamic therapy (PDT) applications (10), none of these fiber optics deliver sensitizer, O₂ and ¹O₂, themselves. Development of a sensitizer and ¹O₂ delivery device would be useful not only for cancer cell treatment but also for bacteria, in cases where local delivery might be advantageous. This study has probed into questions concerning the precise delivery of sensitizer and ¹O₂ to ovarian cancer cells: (1) Does axial force from the probe tip damage ovarian OVCAR-5 cancer cells, (2) can sensitizer be efficiently delivered to the cells, and is fluorine end-capped tip 2 more efficient than conventional tip 1 in doing so, and (3) what is the resultant cell viability, morphology and subcellular localization behavior?

MATERIALS AND METHODS

General information. Pyropheophorbide-a (5) was purchased from Frontier Scientific (Logan, UT). Live/Dead cell assay was purchased from Abcam Inc. (Cambridge, MA). Mitotracker Green FM fluorophore was supplied from Molecular Probes Inc. (Eugene, OR). Fetal Bovine Serum (FBS, 3D cell culture validated) was purchased from Global Cells Solution, Inc. (Charlottesville, VA). Roswell Park Memorial Institute (RPMI 1640) media and penicillin/streptomycin were purchased from Cellgrow, Mediatech Inc. (Manassas, VA). Phosphate buffered saline (PBS) was purchased from Amresco Inc. (Solon, OH). Fetal calf serum (FCS) was purchased from Invitrogen (Carlsbad, CA). Trypan Blue, DMSO, tolune-δ₆ and chlororm-δ₄ were purchased from Sigma-Aldrich (Allentown, PA). Microscopic glass slides (sized: 0.96 x 30 x 100 mm²) were purchased from Corning Inc. (Corning, NY). The hemocytometer used was from Hauser Scientific (Horsham, PA). Round 29 mm glass-bottom
dishes with 10 mm microwells dishes were purchased from In Vitro (Sun- nyvale, CA). Round-bottom plates (96 wells) were purchased from Corning B. V. Life Sciences. Sterile 3 mL vials were purchased from Nalgene (Rochester, NY). Deionized water was purified using a U.S. Filter Corporation deionization system (Vineland, NJ). Porous Vycor glass was purchased from Advanced Glass and Ceramics (Holden, MA) and was dried at 500 °C. A 5 mm diameter × 9 mm length bore was made into the Vycor pieces with a Dremel drill. Images of cells were obtained with a Leica confocal laser-scanning microscope (CLSM, TCS SP2, Leica, Bens- heim, Germany), a Nikon TE2000 fluorescence microscope (Nikon Instruments Inc.) and an Olympus FV-1000 confocal microscope. Fluorescence images were analyzed using MetaMorph software (Molecular Devices, Downingtown, PA). Cell damage was monitored using an AmScope microscope (Irvine, CA). A Newport 1918-C power meter (Newport, Franklin, MA) and a VEGA laser power meter (Ophir Laser Measurement Group, LLC, North Logan, UT) were used for the measurement of energy of the light source. Light was delivered from a 669 nm CW diode laser (model 7404, 506 mW, 2.5 A output, Intense Inc., North Brunswick, NJ).

**FOSD device.** A fiber-optic device with Vycor probe tips 1 and 2 was used as described previously (9). Briefly, pieces of Vycor were “fluorinated” by soaking in 1 × 10⁻³ M 3,3,4,4,5,5,6,6,6-nonafluorohexyltrimethoxysilane and then refluxed in toluene for 24 h. Any nonfluorosilane that was not covalently attached to the Vycor surface was washed away by Soxhlet extraction in methanol for 24 h. Prior to loading of the sensitizer and fluorosilane onto the probe tip, shaping and flattening of the tip was done with a disk grinder and polisher. The probe tips were 5 mm in diameter and 9 mm in length (~0.4 g; 165 mm² total area). The 5 mm diameter front face of the device tip was the operating face for these experiments. The flat shape of the bottom of the probe tip was matched to the flat shape of the OVCAR-5 films and only this front face of the cap (probe tip radius = 2.5 mm; area = 19.7 mm²) was in contact with the cell film. The amount of sensitizer covalently bonded to the bottom plane, not the top plane or the perpendicular cylinder axis of probe tips 1 and 2 was 18 ± 1 nmol and 20 ± 1 nmol respectively (~1.2 × 10¹⁶ covalently attached sensitizer molecules). This was measured by monitoring the sensitizer Soret absorption (415 nm) after liberation from unused probe tips on dissolution with 40 (v/v)% aqueous hydrofluoric acid and extraction with dichloromethane. These tips were affixed to the hollow fiber optic with ethyl cyanoacrylate. A 3 ft long fiberoptic was used which had a Teflon gas flow inner tube running from the distal end to a T-valve. The fiber was connected to a compressed oxygen gas tank via this T-valve (operated at 4 PSI), and delivered 37 nmol O₂ through the probe tip to the ovarian cancer cells per hour. The Teflon tube was surrounded by ~60 excitation fibers randomized in a ring that was encased in a polyvinyl chloride jacket. With the fiber pinned to a translation stage, the diode laser was connected to its proximal end through an SMA con- nector.

**Cells.** OVCAR-5 ovarian carcinoma cells (Fox Chase Cancer Center, Philadelphia, PA) were grown from frozen stocks and maintained in

---

**Figure 1.** Cartoon of the fiber optic-based sensitizer delivery (FOSD) device action leaving behind sensitizer in cells after the alkene was converted into a dioxetane and cleaved apart. Insets: We are looking at the probe bottom, showing the sensitizer being delivered onto the cell film. The flat front face of the device tip has the sensitizer groups extended in the direction of the cell film.

**Figure 2.** Pheophorbide 3 that is photocleaved from the probe tip, and the formation of hydrolysis products: Formic acid, benzyl ester pheophorbide 4, 4-hydroxybenzyl alcohol and parent pheophorbide-α 5.
RESULTS

Not too forceful

To examine whether the axial force exerted by the FOSD probe tip damaged the cells, dynamometer (strain gauge force transducer) measurements were carried out. The equivalent of finger pressure applied in pushing buttons on a computer keyboard (~9 N) (16) does not damage the cells. Forces below 31 N yielded no detectable damage to the cells. However, a force of 44.0 N led to the damage of ~20% of the cells (Fig. 3). The results in these studies were obtained over a range 4.4–44.0 N, but the majority of trials were carried out at 2.2 N.

Sensitizer cleaving “Photo Opportunity”

Table 1 and Fig. 4 show the amount of sensitizer 3 photoreleased into thin films of OVCAR-5 cells using the FOSD device equipped with Vycor probe tip 1 and 2 over 1 h. The front face of the probe tip was in contact with 5.3 × 10^5 OVCAR-5 cells. The amount of sensitizer 3 that cleaved free from the front face of the fluorosilane-coated fiber tip 2 (16.0 nmol) was higher than from 1 (4.6 nmol). Related to this, after photocleavage of the di-O-vinyl ether bridge, we found that lower amounts of sensitizer remain adsorbed onto tip 2 (1.0 nmol) than tip 1 (12.4 nmol).

The photoreleased sensitizer was able to diffuse to cells beyond those in direct contact with the probe tip. The net movement of sensitizer molecules was in the direction of lower concentration and did not depend much on the amount released. The sensitizer diffused laterally by 3.0 ± 0.2 mm for tip 1 and 3.5 ± 0.5 mm for tip 2. The cells in the ring around tip 2 absorbed 9.3 nmol sensitizer (Fig. 5). For the ring of cells around the probe tips, tip 2 led to greater pigmentation per cell (4.5 × 10^-11 mol/3/OVCAR-5 cell) than tip 1 (7.1 × 10^-16 mol/3/OVCAR-5 cell). Previously, the uptake of phophorhede-α 5 was reported to be ~10^-17 mol/5/cell in SK-BR-3 and SK-OV-3 ovarian cancer cells and MDA-MB-468 breast drug-resistant cells (17). The amount of sensitizer used in these previous experiments (0.05 nmol) (17) was some 100–300 times lower than ours. As we expected, in the absence of light and flowing oxygen, the FOSD device showed no detachment of sensitizer molecules.

FOSD photodynamic treatment

As described below, we studied OVCAR-5 cell viability and morphology, and sensitizer localization after treatment with the FOSD device using probe tip 1 and/or 2.

Cell viability and morphology. The results of sensitizer 3 release by the FOSD device into the OVCAR cell film as examined by Trypan Blue staining is shown in Fig. 6. After 1 h of exposure,
The initial cell viability (95 ± 3%) was decreased by 27 ± 3% for probe tip 1 and by 60 ± 5% for probe tip 2. Control experiments with a bare-tipped device devoid of sensitizer molecules sparging O₂ with 669 nm irradiation show viability decreases of < 4%. Other control experiments showed that phorhodisc-a 5, when externally added to the OVCAR-5 cell films, did not decrease cell viability in the dark, as one may expect based on similar results by others (17,18). Bright-field microscopy images of the outward appearance of OVCAR-5 cells prior to and after photooxidation with the FOSD device were compared. The cell morphology images showed noticeable photosensitized degradation after 1 h of exposure (data not shown), where cells were darker in color and about 30% decreased in diameter.

Localization. Subcellular localization of the sensitizer 3 photoreleased from the FOSD device equipped with probe tip 2 was also studied into a monolayer of OVCAR-5 cells (leading to pigmentation of 1.3 × 10⁻¹⁶ mol 3/cell). Figure 7b shows the red fluorescence of sensitizer 3 (excitation/emission = 560/650 nm) that was also localized in the mitochondria based on colocalization with mitotracker green (green fluorescence, Figs. 7c and d). We also observed the localization of phorhodisc-a 5 in mitochondria, 24 h incubation in the absence of the photodynamic treatment. Our results are similar to previous lipophilic sensitizer results (19) and reaffirm phorhodisc localization trends (20,21). For the previously reported 2-(1-propyloxyethyl)-2-dethylpyropheophorhodisc-a, localization was seen in the mitochondria of human pharyngeal squamous cell carcinoma (20). Phorhodisc-a 5 and a phorhodisc diaminobutane polypropylene-imine dendrimer complex were found to localize in the mitochondria in human leukemia (Jurkat) cells in vitro (21). We also saw colocalization of 3 in lysosomes, only after treatment with the FOSD device (data not shown), but did not try to distinguish between the localization effects, instead sought more fundamental insight into device performance.

DISCUSSION
Is it possible to kill cancer cells in vitro with the FOSD device? The FOSD device was tested, is relatively easy to use, and this
There remains a need for a sensitizer and $\text{O}_2$ delivery device to bling device (33). Most, however, are con
terrial pockets or where interstitial PDT is carried out. To eradicate cells from complex sites with precision, such as in bac
c. Magni
geral diffusion distance of the pheophorbide sensitizer(s) in limited ($100 \text{ mm}^2$) for better device perfor
best repel the photocleaved sensitizer
fi
The
fl
Acknowledgements—D.B., D.A., A.G., G.G., I.A. and A.G. acknowledge support from the NIH-National Institute of General Medical Sciences (NIH SC1GM093830). Grant support to T.H. was provided by the NIH-National Cancer Institute (5R01CA160998). We thank Mihaela Minnis for grinding and shaping of glass caps, Stanley Kimani for culturing cells and suggestions and Leda Lee for the graphic arts work. We are also thankful Zhong Wang (Hunter College Bio-Imaging Facility) and Mim Nakarmi (Brooklyn College Physics Department) for use of requisite equipment.

REFERENCES

11. Chakrabarty, A., A. Mallick, B. Haldar, P. Das and N. Chattopadhy

CONCLUSION

The findings with the FOSD device are encouraging. Signifi
Using the FOSD device to eradicate hypoxic cancer cells is a logical next step in the research, as well as the aim for PDT and bacterial applications to kill cells in tightly defined locations in complex 3D systems.

Figure 7. Subcellular localization of sensitizer 3 that was photo-cleaved from device tip 2 into OVCAR-5 cells after (treatment = 1 h) using fluorescence microscopy. (a) Bright-field image, (b) fluorescence image of sensitizer 3, (c) costained with 10 nM mitotracker green and (d) overlay of images b and c. Magnification 100×.


