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REVIEW PAPER

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Singlet oxygen lifetime and diffusion measurements

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ABSTRACT

Introduction. Photodynamic therapy (PDT) is considered to be a promising antitumor methodology due the cytotoxicity of singlet oxygen ($^1O_2$).

Aim. To present singlet oxygen which is highly reactive and decomposes to the ground state rapidly.

Material and methods. Analysis of literature.

Results. This review presents techniques to measure lifetime and diffusion of $^1O_2$.

Keywords. photodynamic therapy, singlet oxygen, diffusion

Introduction

One cancer treatment method that has developed significantly in recent years is photodynamic therapy (PDT). In this method, a patient is given a photosensitizing dye that is intended to accumulate in the target tissue. In the next step, the tumor site is irradiated with light in the visible or near-infrared range. When the photosensitizer (PS) is exposed to light, its molecules are excited and transfer excitation energy to ground state oxygen and singlet oxygen ($^1O_2$) is generated. The resulting singlet oxygen leads to necrosis of the tumor tissue.[1]

PDT is characterized by high efficacy and relatively minor side effects compared to such therapies as radiation therapy or chemotherapy.[2] In the case of PDT therapy, its region of application is very important. In this technique, it is important to provide the sensitizer as close to the cancer cells as possible, which are then selectively destroyed. High reactivity of $^1O_2$ leads to the destruction of healthy cells when it is generated directly in them or at a sufficiently close distance.[3] Due to the short lifetime of $^1O_2$, long-distance travel is impossible.[4] An important role in the diffusion of $^1O_2$ is played by the environment in which it is generated.[5] The most preferred solution would be the selective generation of $^1O_2$ by delivery of light to a specific cellular domain. Currently, in PDT, in addition to diseased cells, their close surroundings may also be illuminated (healthy cells). Therefore, information on the degree of diffusion of $^1O_2$ in cells during its lifetime is very important. That is why it is so important to develop a method that allows measuring and monitoring $^1O_2$. Experiments to determine the value of the intracellular

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lifetime of $^1O_2$ have significant value for the PDT method. Previous studies have established that the intracellular lifetime of $^1O_2$ and the distance traveled by $^1O_2$ during this period is severely limited due to intracellular viscosity that hinders translational movement.[4] In this review, examples of lifetime and singlet oxygen diffusion in different media are presented.

Measurements of lifetime and diffusion of singlet oxygen

A $^1O_2$ measurement lifetime measurement is difficult due to its very short lifetime and requires the use of advanced equipment. By measuring the lifetime of singlet oxygen we are able to determine its diffusion distance.[6] The range d, in which $^1O_2$ can exist, is limited by its lifetime τ, which correlates with its diffusion coefficient D (Equation 1).[7]

$$d = \sqrt{2\tau D}$$

Equation 1.

Table 1. Lifetimes of $^1O_2$ in various solvents

References	Solvent	$^1O_2$ lifetime (μs)
Rodgers M. A. J.[9]	H_2O	4 ± 2
Adams D. R. & Wilkinson F.[12]	D_2O	30 ± 10
Merkel P. B. & Kearns D. R.[10] and Long C. A. & Kearns D. R.[11]	H_2O:CH_3OH, 1:1	3.5
	D_2O:CH_3OH, 1:1	11
	$CHCl_3$	60 ± 15
	CS_2	200 ± 60
	$CDCl_3$	300 ± 100
	C_6F_6	600 ± 200
Adams D. R. & Wilkinson F.[12]	$(CD_3)_2CO$	640
Merkel P. B. & Kearns D. R.[10] and Long C. A. & Kearns D. R.[11]	CCl_4	700 ± 200
	CCl_3F / (Freon 11)	1000 ± 200

Data available in the literature determine the range of travel of $^1O_2$ in water at room temperature at d = 125 nm.[8] The lifetime of $^1O_2$ in cells is shortened by reaction with cellular molecules. Redmond and Kochevar have shown in their research that chemical reactions of singlet oxygen with protein amino acids, nucleic acids or with unsaturated lipids reduce the lifetime of singlet oxygen.[8] The generation of singlet oxygen can take place in various types of solvents. The solubility of the substrate and sensitizer as well as the properties of the solvent used has a significant effect on the life expectancy of $^1O_2$. Table 1 shows lifetimes of $^1O_2$ for various solvents.

In PDT, it is the lifetime of $^1O_2$ that determines how excited the oxygen molecule can diffuse from the place where it was created. For live cells, the *D* value for molecular oxygen is around $1.4 \times 10^{-5}\,cm^2s^{-1}$, hence the diffusion length under these conditions is in the order of nm.[13] Currently available data on the oxygen diffusion coefficient indicate that subcellular domains that tend to be quite viscous have a significant impact on its average/apparent value.[14] Table 2 presents examples of studies on the diffusion of singlet oxygen in cells.

Induction of minimal damage that arises during PDT is often tolerated by healthy cells located in close proximity to the site of $^1O_2$ formation; it may even lead to stimulation of their growth. The photosensitizers used play an important role in this case.[13] This shows that the diffusion distance of $^1O_2$ is very important in the case of PDT. A study conducted by Ogilby et al. in 2006 involving the detection of singlet oxygen from single cells showed that the lifetime of $^1O_2$ may be longer than commonly thought. It also means that singlet oxygen can diffuse over long distances, including through the cell membrane to the extracellular environment.[21] Similar results were obtained by Hatz et al. in studies measuring the lifetime of singlet oxygen in a single cell.[22] These studies show that in a living, functioning cell containing water, the lifetime of singlet oxygen is about 3μs.[22] In turn, in a study conducted by Kuimova et al. monitored the time-resolved luminescence decay of $^1O_2$ after production by the sensitizers chlorin (Chl) and 5,10,15,20-Tetrakis(N-methyl-4-pyridinio)-21H,23H-porphine (TMPyP) that were localized in different domains of the living cells.[6] The obtained data indicated that both the lifetime and rate constant for $^1O_2$ quenching depends on the photosensitizer. In addition, these studies have shown that despite the relatively long intracellular lifetime, due to the heterogeneity of the cell, $^1O_2$ does not diffuse at long distances from the place of its production. The authors of this study also pointed out that high intracellular viscosity has a significant impact on this.

A diagram showing the operation of PDT in clinical use is shown in Figure 1. Under the influence of laser light, we observe the production of $^1O_2$, which in addition to destroying cancer cells, may diffuse into neighboring cells causing healthy tissue damage.

Studies related to the effects of singlet oxygen on lipid membranes are often reported.[23,24] In many studies, diffusion distances of $^1O_2$ are calculated based on studies carried out on model lipid membranes that are protein-free. The studies conducted by Pooler aimed to check whether there are differences in $^1O_2$ diffusion in the case of lipid domains and band protein 3.[25] The results obtained showed that the diffusion of singlet oxygen from both locations is at the same level and no significant differences in the results were ob-

Table 2. Measurements of diffusion distance of singlet oxygen in different media

References	Materials and Methods	Media	Distance
Hatz S. *et al.*[4]	the single cell experiments were performed using a microscope with the focused output of a pulsed fs laser as the excitation source; O_2 (a^1 Δg) was monitored using a cooled photomultiplier tube operated in a photon counting mode; the sensitizer used in these experiments was 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP)	cells	60–159 nm
Moan J. & Berg K.[15]	NHIK 3025 cells were incubated with Photofrin II (PII) and/or tetra (3-hydroxyphenyl)porphyrin (3THPP) and exposed to light at either 400 or 420 nm	cells	10 - 20 nm
Redmond R.W. & Kochevar I.E.[8]	decay kinetics and diffusion distance of singlet oxygen in aqueous solution	water	~ 125 nm
Egorov S.Y. *et al.*[16]	luminescence component with a lifetime of about 1μs in yeast cells,	yeast cells	≤ 0.07 μm
Sokolov V. *et al.*[17]	aluminum phthalocyanines were adsorbed to only one interface of planar lipid bilayers	lipid membrane	~ 100 nm
Krasnovsky A.A.[18]	diffusion distance from the generation site in chloroplas thylakoids	chloroplas thylakoids	~ 5.5 nm
Skovsen E. *et al.*[5]	in the nucleus of the cell, experiments were performed in which the cell was exposed to bovine serum albumin (BSA); in D_2O-based experiments, the medium surrounding the cell contained 0.77 mM BSA; the distance traveled by singlet oxygen in 6 μs was measured	cells	~ 268 nm
Baier J. *et al.*[19]	incubated the HT29 cells with Photofrin or ATMPn, the photosensitizer molecules were located in the cellular membranes (fluid, heterogeneous mosaics of proteins and lipids)	cellular membranes	~ 3 μm
Dysart J.S. *et al.*[20]	in vitro experiments were performed in which MatLyLu (MLL) cells were incubated in Photofrin	cells	~ 0.05 μm

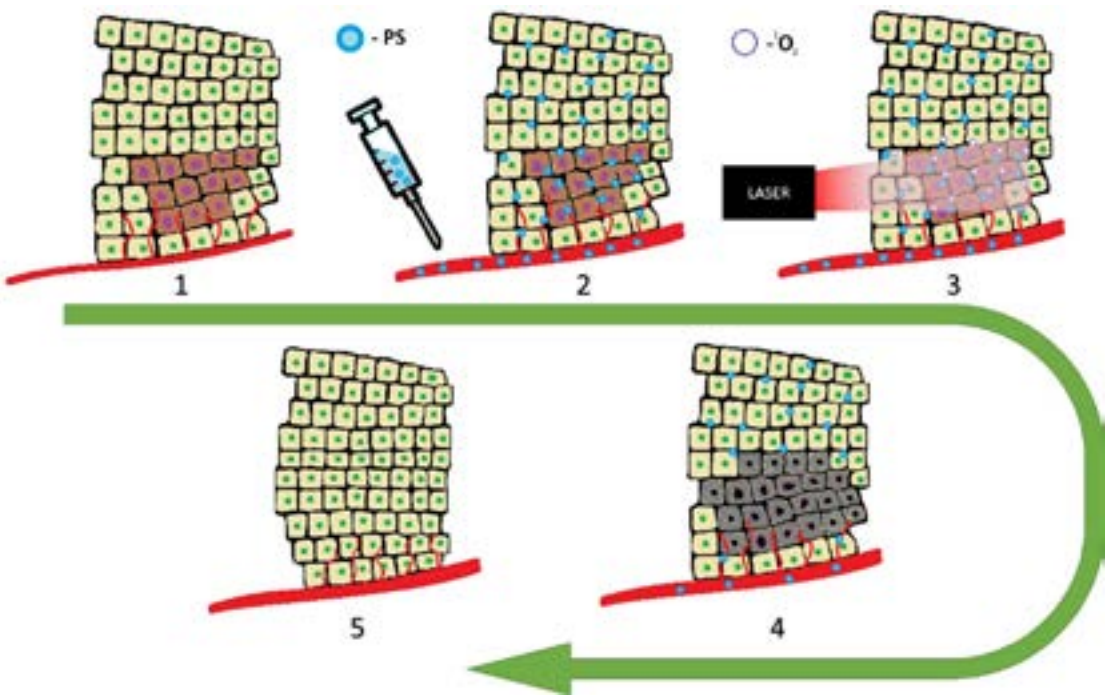

Fig. 1. A schematic illustration of photodynamic therapy. 1 – normal and cancer cells; 2 – PS administration and accumulation in cells; 3 – irradiation and $^1O_2$ production; 4 – tumor necrosis and damage to neighboring healthy cells; 5 – tissue regeneration

served. Moan et al. arrived at completely different conclusions and discovered that $^1O_2$ was unable to diffuse at sufficient distance to trigger the effect of Photofrin molecules in cell membranes, despite the fact that the calculations showed that the distance between adjacent molecules was small enough for this effect to occur.[26] These studies suggest that proteins shorten the lifetime of $^1O_2$, resulting in a reduction in diffusion distances inside cell membranes.

Conclusion

This review includes studies on the lifetime and the diffusion of singlet oxygen. The lifetime of singlet oxygen and its diffusion distance are interrelated. Diffusion $^1O_2$ depends not only on the photosensitizers used, but above all on the medium in which ROS production is to take place. All studies have one conclusion that is a critical aspect in photodynamic therapy which is that the photosensitizer should be as close as possible to the cancer cell.

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