

Yeast, a convenient model system to study the biological effects of laser irradiation

Hideo Tashiro and Galina Lazarova
Photo-Biology Group, Photodynamics Research Center

The wide application of lasers in medicine and biology requires intensive studies on the irradiation effects at cellular and molecular levels. Our investigations using yeast cells as model system are directed to modification of the cellular response to laser irradiation by chemical and physical factors. Some experimental procedures developed, recent results and future plans are presented in brief.

There are two major areas of application of lasers in medicine: photodynamic cancer treatment¹⁾ and surgery.²⁾ In the photodynamic treatment irradiation is usually in the visible range, whereas in the surgery infrared or shortwave irradiation is used.

Yeasts are an established and convenient model applied in numerous photobiological studies as an alternative to cell cultures and experimental animals.³⁾ The test strains we use are *Saccharomyces cerevisiae* XS2316 and *Kluyveromyces fragilis* DSM 7238. The latter strain has the advantage to be thermotolerant and thus to be adequate for experiments at the standard physiological temperature of 37 °C or even higher (up to 42 °C) providing information on the effects studied in shorter time than in the case with *Saccharomyces cerevisiae*.

The application of photodynamic effects in treatment of several diseases results in growing interest on modification of the photosensitized lethal action by physical and chemical factors. Many investigations are directed toward finding agents enhancing photodynamic reactions or protecting the cells against photodynamically induced killing. In an attempt to modify the cellular response to photodynamic damage we chose Amphotericin B, a widely used fungicidal drug which is well known to inactivate yeast cells via disruption of the membrane barrier.⁴⁾ Low concentrations of Amphotericin B affect membrane permeability and can be exploited to increase the therapeutic efficacy of antibiotics and antitumor agents which normally penetrate poorly or not at all into eucaryotic cells. In this connection it is reasonable to expect a synergistic effect of Amphotericin B in photodynamic treatment where the photosensitizer penetration through the membrane into the cell is the limiting factor.

Our study initially designed to prove that the considerations stated above revealed an effect opposite to the expectations: Amphotericin B did not act synergistically with the photosensitizers, on the contrary it exerted a strong protective effect.⁵⁾ The protection was observed both with photosensitizers affecting the cell membrane (Rose Bengal, Toluidine Blue, Methylene Blue) as well as with 8-MOP exerting predominantly genotoxic effects. Figure 1 shows the protective effect of Amphotericin B under the conditions of photodynamic cell damage using a frequency-doubled Q-switched Nd:YAG laser at 532 nm and Methylene Blue as the photosensitizer.

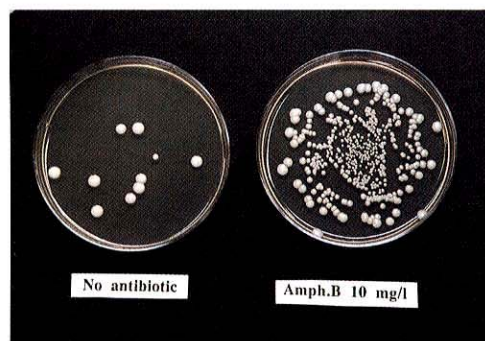


Fig. 1. Under the photodynamic damage performed by a Q-switched Nd:YAG laser emitting at 532 nm with Methylene Blue as a photosensitizer, the survival of the yeast cells is enhanced in the presence of Amphotericin B.

Our plans for further research are directed to the investigation of killing, mutagenic and membrane damaging effects of vacuum-ultraviolet (VUV) irradiation. Yeasts have been already used in order to elucidate the mechanism of cell destruction by VUV. Thorough investigations have been performed on the effect of continuous wave VUV (145 to 190 nm) irradiation on yeast using a synchrotron or deuterium discharge lamp.⁶⁾ The numerous data collected indicate that the cell membrane damage is a primary cause for the VUV killing effect. The lack of DNA damage of yeast cells irradiated by VUV is explained by its low penetration to the cell center where DNA is localized.⁷⁾ On the other hand, by using 193

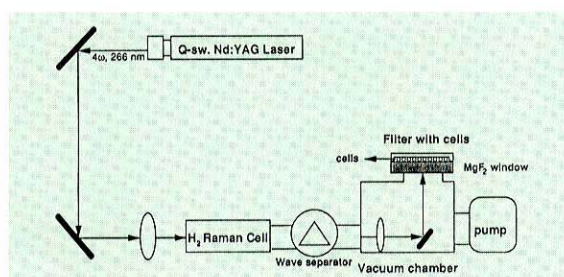


Fig. 2. Experimental set-up for the laser vacuum-ultraviolet irradiation of yeast cells.

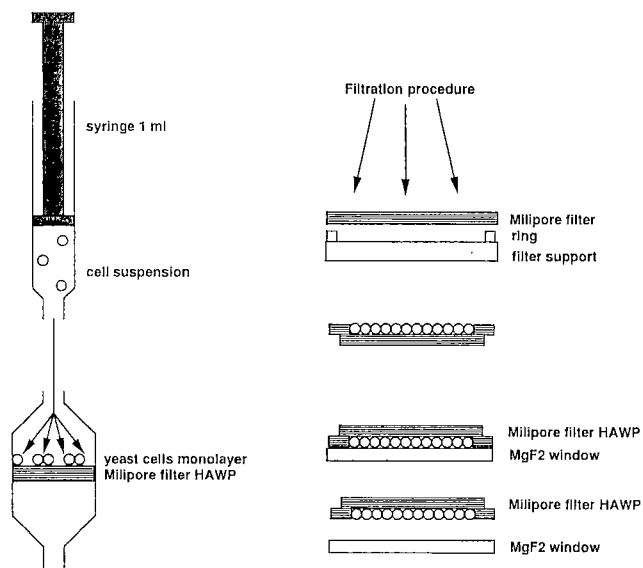


Fig. 3. Preparation of a sample for the vacuum-ultraviolet irradiation. The yeast cells are loaded on a Milipore filter in monolayer with simultaneous deformation of the filter resulting in a plate-shaped surface. This procedure prevents the adherence of the cells to the optical window.

nm excimer laser, DNA damage was proven to be a very important factor causing the yeast cell death.⁸⁾ It is then not clear whether the discrepancy can be attributed to the pulsed nature of the irradiation in the latter case.

Our investigations on the mechanism of yeast damage are performed with a new irradiation source,⁹⁾ which we have developed based on the Nd:YAG laser and anti-Stokes Raman up-conversion technique (Fig. 2). Due to the low penetration of the VUV-beam in air, a close contact between the irradiated cells and the optical Mg_2F window has to be provided. To prepare the samples for irradiation we attach the yeast cells to Milipore filters (Fig. 3). The procedure is performed in such a way that after the filtration the loaded filters become plate-shaped.

The approach presented here will make it possible to obtain new information concerning the biological effect of pulsed VUV irradiation.

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