

Review Article

Microscopic Diagnostics and Mapping of Cytophysiological Effects of Reactive Oxygen Species during Microwave Exposure of Living Tissues

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Abstract

A specialized installation has been developed for microscopic study of the Reactive Oxygen Species (ROS) formation during microwave irradiation of biological samples with automated control / mechanized tube and real-time data acquisition. The above installation can be used in biomedical practice for: standardization or certification of the microwave sources; testing of the potential antioxidants that protect tissues from ROS-induced effects; testing fluorescent sensors for ROS; analysis of ROS localization and distribution in various tissues in order to establish specific pharmacophysiotherapeutic and toxicological localizations of ROS in different topographic-anatomical zones. The paper pays special attention to the singlet oxygen produced by the samples upon microwave treatment, as a physiologically active and highly reactive agent.

Introduction

The problem of singlet oxygen is crucial for biomedical physics, clinical biochemistry, and molecular medicine, since singlet oxygen is a highly reactive low-molecular-weight agent that damages membranes [1], cytoplasmic proteins [2], nucleic acids [3] (or mediating the effects of both organic [4] and inorganic [5] toxic agents on them). Typically, membrane lipids that protect against singlet oxygen effects during photo-induced damage [6,7] are not able to eliminate the effects of its volumetric exposure when using other, more physiologically active sourced of the singlet oxygen generation, especially non-photobiological agents capable of penetrating deep into the cell.

In particular, it is known that singlet oxygen is formed under the microwave radiation and in a microwave discharge [8,9], obtained in such experimentally accessible sources as chemical (for example, oxygen-iodine [10]) lasers, gas-discharge lamps [11], radio frequency or microwave plasmotrons [12]. It can be assumed that the effect of such sources on biological tissues, not associated with the photochemical processes, is determined by the centimeter or decimeter waves, resulting in nonspecific oxidative stress at the cellular level. There are medically relevant reports of oxidative stress caused by

microwave frequencies typical of microwave ovens, cellular communications, and wireless networks based on the IEEE 802.11 (Wi-Fi) communication standard [13]. The phenomena induced by these frequencies are histochemically significant and can be diagnosed microscopically as histopathological changes [14]. Accordingly, there is a need for designs that make it possible to simultaneously effect the cells or tissues with the centimeter / decimeter frequencies and microscopically observe the changes induced along with mapping their lability to the oxidative stress. The development of such a system would make it possible not only to observe the above changes, but also to study the effectiveness of the potential protectors, such as selenium and L-carnitine [15,16]. In this paper we present the design of an installation capable of performing these functions.

Installation design and analysis capabilities

The proposed version of the installation design is based on the previously published concept of analyzing the ROS levels in tissues during irradiation with the chemical calibration based on the background measurements in the environment near the irradiation source [17,18]. This is necessary because determining individual reactive oxygen species is challenging due to their co-localization in the cell under nonspecific

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oxidative stress and the shared mechanisms of their effects on the cell (in the case of microwave irradiation this is manifested in the unity of action of ozone, hydrogen peroxide and other ROS and the direct effects of microwave radiation [19,20]). The prototype apparatus assembled is shown in Figure 1, while a chemometric system for monitoring ROS production in the environment, which is the subject of a separate work, is not shown.

The proposed design consists of a column in which a microscopic tube is placed, moved using the stepper motors (equipped with an opaque illuminator and an Ocean Optics fiber optic spectrofluorimeter mounted on a turret attachment) and on which an optical detection unit, a pulsed power supply, and a magnetron module with a pre-exposure camera are located. In the latter a sample is irradiated and then observed. The installation also includes an exposure control panel and a computer with software for data acquisition and processing. In the original version, the system operates at the relevant frequency of 2.45 GHz. Power and pulse modes are controlled by a panel unit, functionally similar to that of a microwave oven.

Theoretically, based on the medical physics data, it may also be appropriate to perform measurements at other frequencies (for example, 900 MHz, 930 MHz, and 1.8 GHz), at which the ROS generation and oxidative stress phenomena are recorded [21-23] (while at 1.9 GHz these data are not confirmed [24]). For this purpose, the installation provides the possibility to replace the magnetron and the exposure control panel. A noise meter was selected for this wavelength range, intended for electromagnetic noise protection measurements, since it is known that with an increase in the ROS concentrations and the DNA damage induced by them [25], electromagnetic noise can inhibit this fragmentation, leading to decreased ROS concentration [26].

The measurements are carried out in conditions protected from light (the gateway is closed at the time of exposure) in order to exclude non-thermal effects not envisaged by many authors (but also acting on the tissues) and to indicate only the response to the microwave exposure, in accordance with the work [27]. It should be noted that the authors were unable to completely standardize the microwave radiation by the wavelength, since at microwave frequencies, the magnetron operates across a range of 2.3 to 2.7 GHz and it is almost impossible to level out the parasitic modulation of the magnetron within its operational voltage range (3.5 - 4 kV). Otherwise, this installation is standardized to the levels where, according to literature, fluctuations in readings do not alter the nature of observed physiological effects. The design incorporates double shielding, ensuring complete safety for the operator. This installation can also implement fluorescence mapping techniques using specific ROS-sensitive dyes and fluorescent sensors [28].

In this case, the most oxidized areas in the sample may

not directly correspond to ROS-generating locations due to the different oxidability of the macromolecular cell components, organelles, and the permeability differences for different compartments. In this regard, to objectively compare singlet oxygen generation efficiency in different sample areas, it is necessary to use not the indirect methods of ROS determination by the oxidation products, but the direct (in particular, fluorescent) methods for detecting reactive oxygen species *in situ* during separation, which requires the development of the control algorithms for the mechatronic subsystem of the installation corresponding to the specific exposure conditions.

In other words, in a number of experimental techniques, this installation will make it possible to map not the redox status of the cell as a final chemical outcome, but rather its lability or resistance, reflecting the physiological status, oxidability of a particular area depending on the microwave irradiation parameters. This is a qualitatively new form of functional-morphological and clinical-histochemical interpretation of *in situ* analysis, taking into account the form and parameters of the inductor, which were not fully described in this study [29].

Since microwaves at a wavelength of 2.45 GHz provide heating of the biological structures, and the heating of a particular structure depends on the water content, high-resolution thermography can be performed over an extended dynamic range in this installation - the so-called NIR-HDRI thermography (or bolometric measurements using enthrakometers [30]), with the co-localization analysis of the ROS and water content and thermographic characteristics mapped under the microwave irradiation. Accordingly, in the dynamic version, the technique proposed can be partially used to analyze diffusion processes and heat and mass transport. This may be useful not so much for monitoring the living cells or tissues, which are typically destroyed during such sample preparation, but for monitoring vesicular transporters of the pharmaceuticals at the preclinical stage.

It is noteworthy that the first installation shown in Figure 1 was not optimal in terms of the ratio of the chamber size and the

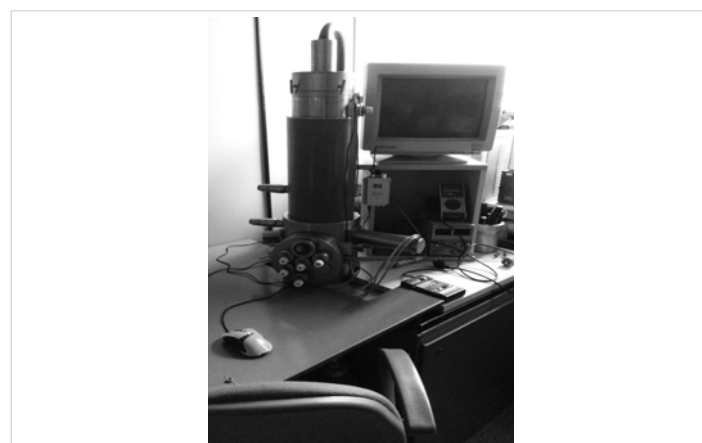


Figure 1: Modified SEM column for microwave, optical, and photochemical investigations of the living tissues.



wavelength used, that is: the working chamber as a resonator formed suboptimal configurations of standing waves with the multiple reflections from the walls, therefore producing overheating, heating gradients, and operational instability due to the insufficient chamber volume. Therefore, while formulating the priority, we cannot yet suggest the prospects for immediate introducing of this tool into the widespread biomedical practice, since its numerical optimization is required using HFSS (from ANSYS) or its analogs.

Conclusion

Thus, this is the first system designed to study ROS production depending on the irradiation parameters; to determine co-localization with other physicochemical parameters related to irradiation; to perform microwave heating thermography and to quantify analyte concentration; to employ fluorescent techniques using fiber optic spectrofluorimetry in order to perform direct detection of the ROS generated.

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