Interaction of low-temperature atmospheric pressure plasma jet mixed with argon and air with living tissues

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Abstract. The paper presents the results of spectral-fluorescence and diffuse-optical studies of biological tissues when exposed to a low-temperature atmospheric pressure plasma in a mixture of air with argon with an exposure of up to 10 minutes. Potential fluorophores were identified and the coefficients of optical absorption and transport scattering of biological tissues were determined. The analysis conducted allows to conclude that plasma probing leads to significant inhibition of cellular respiration and the development of coagulation effects in biological tissues.

1. Introduction

Today, one of the promising and rapidly developing areas of radiation medicine is plasma medicine demonstrating high therapeutic benefits in the treatment of superficial and ulcerative defects of the skin and internal organs (see, for example, [1–3]). At the same time, the review of publications in the field of plasma medicine indicates that the results so far obtained are mostly qualitative in nature and do not contain information about the processes of interaction of plasma with living systems [2–5]. Further development and adoption of this advanced technology requires thorough understanding of the complicated physical and biochemical mechanisms of the complex interaction of plasma with living tissues, cells and microorganisms.

From the point of view of diagnostics informative value, the combined method of stationary laser-induced fluorescence (LIF) spectroscopy and diffuse reflectometry is of great interest (see, for example, [6, 7]). This approach allows to conduct non-invasive studies of living objects and provides information about the physiological, structural, morphological and biochemical properties of in situ samples [8, 9].

The aim of this work is to conduct spectroscopic research of low-temperature plasma influence on living tissues. For this purpose, the fluorescence and diffuse reflectance spectra were measured, and the absorption and transport scattering coefficients of intact and irradiated living tissues were determined. On the basis of the results, a preliminary estimation of the dynamics of the most important biochemical and morphofunctional parameters of living tissues is given.

2. Materials and methods

The research of the dynamics of the spectral-optical properties of living tissues, when exposed to a low-temperature atmospheric-pressure plasma jet (APPJ), was carried out on 18 samples of normal tissue of a rat liver in vivo. The same areas of liver tissue, not exceeding the sizes of 10.0 × 10.0 × 5.0 mm, were subjected to plasma probing and spectral studies.
Probing the samples with the APPJ in a mixture of air and argon was carried out for 10 minutes. The samples were positioned perpendicular to the jet at a distance of 1.5 cm from the end of the plasma waveguide. The argon flow was fixed at a value of 0.32 l/min, at which the plasma torch reached its greatest length. Preliminary measurements of the APPJ temperature showed that the gas temperature did not rise above 2°C relative to room temperature, which excluded thermal influence on living tissues. A detailed description of the experimental setup for producing the low-temperature APPJ is given in [10].

Immediately after plasma probing, the fluorescence $F(\lambda)$ and diffuse reflection $R_d(\lambda)$ spectra of living tissues were measured using a fiber-optic system for delivering and collecting optical radiation. A block diagram of this experimental setup is given in figure 1.

![Figure 1. Scheme of obtaining atmospheric pressure plasma jet mixed with air and noble gas.](image)

The plasma was created by a barrier discharge in a quartz tube with an external diameter of 7 mm and an internal of 1 mm. One of the electrodes was a metal rod with a diameter of 0.5 mm, located inside the quartz tube, at a distance of 60 mm from it; the second electrode was installed outside the quartz tube and was a grounded metal ring 10 mm wide. Its diameter coincided with the outer diameter of the quartz tube. Voltage pulses with an amplitude of up to 15 kV and a duration of about 500 ns were applied to this gas-discharge system.

![Figure 2. Characteristic spectral distribution of the APPJ radiation intensity (a), radiation intensity (b) and photo of the jet taken across the plasma torch movement.](image)

Figure 2 shows the characteristic spectral and optical parameters of the APPJ in a mixture of air and argon.

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The excitation of the fluorescence spectra was carried out by the radiation of LP603 optical parametric generator complete with LQ529 pump laser (Solar Laser System, Belarus), at wavelengths of 355 and 400 nm, and the diffuse reflection coefficient – by the radiation of a AvaLight-DH-S-BAL deuterium/halogen lamp (Avantes, The Netherlands).

A Y-shaped measuring probe consisting of two hoses (transmitting and receiving ones) and a contact catheter with coaxially placed fiber-optic channels was used as a fiber-optic system. Fiber-optic cables forming the central channel of the probe with a diameter of 600 μm were intended to bring the exciting radiation to the sample, while the optical fibers located on the periphery formed a recording channel with a diameter of 1.5 mm and were used to collect photo signals and transmit them to the spectrometer.

Spectral analysis of the photo signals was carried out using an automated MS3504i monochromator/spectrometer (SOL-Instruments, Belarus) and a HS-101 (HR)-2048 × 122 CCD camera (Hamamatsu, Japan) combined with a personal computer.

Three series of measurements were performed for each sample of biological tissues \( F(\lambda) \) and \( R_d(\lambda) \). The final result for the samples investigated was determined by averaging the serial measurements.

To analyze the diffuse reflection coefficient \( R_d(\lambda) \) and determine the coefficients of optical absorption \( \mu_a(\lambda) \) and reduced scattering coefficient \( \mu_s'(\lambda) \) of biological tissue, we used the technique described in [11].

### 3. Results and discussion

Typical stationary fluorescence spectra - \( F(\lambda) \) of biological tissues in the normal condition and under AAPJ probing with an exposure of 10 minutes are shown in figure 3. In this case, curve 1 corresponds to fluorescence spectra obtained upon excitation at wavelengths \( \lambda_{ex} = 355 \text{ nm} \), and curve 2 corresponds to excitation \( \lambda_{ex} = 400 \text{ nm} \).

A comparison of the results obtained with well-known literature data (see, for example, [7–9, 12]) made it possible to establish that the fluorescence spectra of the first group (curve 1), which are characterized by the presence of the main maximum at the wavelength of 475±3 nm and spectral components near 531±2 and 593±5 nm, are mainly formed by the glow of dehydrogenase coenzymes (reduced form of nicotinamide adenine dinucleotide (phosphate) – NAD(P)-H and the oxidized form of flavoproteins – FAD\(^+\)) as well as asporphyrin complexes. Moreover, the fluorescence of the last two fluorophores is most visibly reflected in the \( F(\lambda) \) spectra of the second group (curve 2), forming a glow contour with an intense band in the wavelength range of 490 - 520 nm and extrema at 593 ± 2 and 635 ± 2 nm.

Typical spectra of diffuse reflection - \( R_d(\lambda) \), absorption coefficient – \( \mu_a(\lambda) \) and reduced scattering coefficient – \( \mu_s'(\lambda) \) for biological tissues in the normal condition (A-B) and under AAPJ probing with the exposure of 10 minutes (C-D) are shown in figure 4. As can be seen, the diffuse reflection coefficients of the biological tissues investigated have similar spectral contours, reaching their minimums in the area of blood absorption, and a section of high-intensity in the area of the therapeutic window. Meanwhile, plasma probing of the specified duration leads, as in the case of fluorescence spectra, to an increase in the reflection coefficient of up to 1.5 times.

Applying the standard deviation method as a statistical analysis of fluorescence spectra, allowed to establish that the maximum spread in \( F(\lambda) \) values did not exceed 10–15 % over the entire spectral range of our research.

The effect of low-temperature atmospheric pressure plasma on liver tissue leads to a change in both the intensity and shape of the spectral contours of fluorescence. A comparative analysis of the results obtained by comparing the spectral coefficients defined as the ratio of the luminescence intensity maxima at the wavelengths of endogenous fluorophores - \( \kappa_1 = \frac{I_{F}^{355}}{I_{F}^{475}}, \kappa_2 = \frac{I_{F}^{520}}{I_{F}^{475}} \) and \( \kappa_3 = \frac{I_{F}^{595} + I_{F}^{635}}{2I_{F}^{475}} \), revealed the following. Statistically, the data on the spectral indices k1-k3 for biological tissues in the normal condition and under AAPJ probing with an exposure of 10 minutes are shown in figure 5. As can be seen from figure 3 and 4, against the background of a general increase in the fluorescence intensity of up to 1.5 times, a 15–20 % decrease in the luminescence of flavin and porphyrin groups is
observed. This fact is evidence in favor of a slowdown in redox processes in irradiated biological tissues, which confirms the therapeutic effect of low-temperature atmospheric pressure plasma [4, 5, 13].

Figure 3. Stationary fluorescence spectra – \( F(\lambda) \) of biological tissue before radiation (A) and after radiation of APPJ (B). 1. – Fluorescence spectra obtained at a wavelength of \( \lambda_{ex} = 355 \) nm; 2. – Fluorescence spectra obtained at a wavelength of \( \lambda_{ex} = 400 \) nm. Vertical lines are a standard error.

Figure 4. Diffuse reflectance spectra – \( Rd(\lambda) \), absorption coefficient – \( \mu_a(\lambda) \) and reduced scattering coefficient – \( \mu_s'(\lambda) \) of biological tissues before radiation (A-B) and after radiation by APPJ (C-D). Circles are the reconstruction of the spectrum of diffuse reflectance coefficient – \( Rd_{Calc} \), line is the approximation of reduced scattering coefficient – \( \mu_s'_{calc} \).

An analysis of the spectral dependence of absorption coefficients – \( \mu_a(\lambda) \) of liver tissues suggests that the shape of their spectral contour (figure 4B and 4D) is formed by a complex of endogenous chromophores, which, first of all, include oxy- and deoxyhemoglobin with absorption bands at wavelengths of 350 ± 10, 418 ± 5, 545 ± 5 and 577 ± 5 nm. At the same time, the presence of other spectral components is probably caused by the availability of bilirubin and biliverdin in living tissues with absorption bands at wavelengths of 280±5, 410±5 and 630±15 nm, as well as lipids and water – 760±5 and 970±5 nm [12]. In comparison, the spectral contour of the reduced scattering coefficient – \( \mu_s'(\lambda) \) is a smooth curve that gradually descends towards longer wavelengths, whose intensity and slope is determined by the density and size of the scattering particles.

The effect of low-temperature plasma with a slight decrease in the absorption coefficient (within 10%) relative to the norm leads to an increase in the transport scattering coefficient up to 2 times with
an increase in the slope of the curve.

An analysis of the scattering anisotropy index of biological tissue by its approximating to a power function \( \mu'_s(\lambda) = a\lambda^{-b} \) (where \( \lambda \) is the wavelength in nm, \( a \) is a function of the density of scattering particles, and the parameter \( b \) determines their average size) [14] allows to predict the transport scattering coefficient with a function that for normal biological tissues takes the form: 

\[
\mu'_s(\lambda)_{calc} = 34.677\lambda^{-0.571} + 4.169 \times 10^9\lambda^{-3.622}.
\]

The fact of approximation through the combination of two power functions indicates the formation of the \( \mu'_s(\lambda) \) spectrum with at least two types of particles. Moreover, the first term of this function with the wave exponent \( b \sim 0.57 \) is responsible for light scattering caused by sufficiently large particles (Mie scatterers) that can be nuclei and membranes of hepatocytes, bundles and plexuses of connective tissue fibers and other large components of biological tissue. The second term with the wave exponent \( b \sim 3.8 \) corresponds to small particles (Rayleigh's), such as mitochondria and other elements of the cytoplasm of hepatocytes, individual collagen fibers of the tissue stroma, supramolecular complexes, etc.

![Figure 5. Histogram of k₁-k₃ spectral indices](image)

For irradiated biological tissues, the approximating function takes the form: 

\[
\mu'_s(\lambda)_{calc} = 33.512\lambda^{-0.548} + 9.518 \times 10^8\lambda^{-3.937}.
\]

A comparative analysis of these functions shows that with CAP impact, the effective size and density of Mie scatterers in living tissues change only slightly, while the size of Rayleigh particles decreases and their density increases.

The detected morphofunctional changes in biological tissues may indicate the development of coagulation effects. Apparently, during 10-minute plasma probing, there occurs a destruction of the histostructure and architectonics of the hepatic parenchyma, associated with a decrease in the size of the structural elements of the cytoplasm, wrinkling and coagulation of the ultra-structures of hepatocytes and tissue stroma. However, for a more detailed investigation of this relationship, an additional histomorphological and histochemical analysis should be conducted, which is beyond the scope of this paper and will be taken into account in subsequent studies.

In addition, the detected change in the optical properties of liver tissues caused by the coagulation processes under the impact of APPJ may also be the source of a significant increase in the fluorescence intensity of irradiated living tissues. This effect is well known when performing laser interstitial thermotherapy of malignant neoplasms and is one of the factors for monitoring the degree of coagulation of the living tissues (see, for example [6, 15]).

However, it should be noted that for a more detailed studies of the detected biochemical and morphofunctional effects requires additional spectroscopic studies, as well as microscopic histomorphological and histochemical analysis, which is beyond the scope of this work and will be taken into account in the future studies.

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4. Conclusion

Thus, summarizing the results of the study of the effect of APPJ on the spectral-optical properties of biological tissues, we can draw the following conclusions:

1. The fluorescence spectra $F(\lambda)$ of the studied living tissues are formed by the glow of coenzymes of NAD(P)$\cdot$H and FAD$\cdot$ dehydrogenases, as well as a complex of endogenous porphyrins. At their radiation wavelengths of 475 ± 3 nm, 531 ± 2, 593 ± 5 and 635 ± 2 nm extremes are observed. With a general increase in fluorescence intensity up to 1.5 times, a change in the shape of $F(\lambda)$ spectral contour caused by plasma probing is associated with a decrease in the luminescence intensity of flavin and porphyrin groups by 15–20 %, which testifies to the inhibition of cellular respiration in irradiated biological tissues.

2. The spectra of diffuse reflection coefficient $R_d(\lambda)$ contrasted with backscattering are characterized by the presence of minimums formed by the absorption coefficient $\mu_a(\lambda)$ of hemoglobin at 350±10, 418±5, 545±5 and 577±5 nm, bilirubin and biliverdin at 280±5, 410±5 and 630±15 nm, and lipids and water at 760±5 and 970±5 nm. At the same time, a 1.5-fold increase in the $R_d(\lambda)$ coefficient observed during plasma exposure is mainly caused by an increase in transport scattering coefficient $\mu'_s(\lambda)$.

3. The analysis of the transport scattering coefficient of the biological tissues investigated points to a change in their structural and morphological properties caused by CAP impact with an exposure time of 10 minutes, which may indicate the development of coagulation effects.

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