Spectroscopic techniques to study the immune response in human saliva

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Abstract. Studies of the immune response dynamics by means of spectroscopic techniques, i.e., laser correlation spectroscopy and fluorescence spectroscopy, are described. The laser correlation spectroscopy is aimed at measuring sizes of particles in biological fluids. The fluorescence spectroscopy allows studying of the conformational and other structural changings in immune complex. We have developed a new scheme of a laser correlation spectrometer and an original signal processing algorithm. We have suggested a new fluorescence detection scheme based on a prism and an integrating pin diode. The developed system based on the spectroscopic techniques allows studies of complex process in human saliva and opens some prospects for an individual treatment of immune diseases.

1. Introduction

Antibacterial properties of a human body play an important role in protection of an organism against an infection [1]. The ability to kill microbes is an important parameter in assessment of the immune status of the person especially at pathology [2]. Consequently, it is important to estimate activity of humoral and cell mediated immune responses. The humoral immunity works through various substances like blood plasma, saliva and glandula secretions which are capable to suppress reproduction of microbes [3]. Among these substances, antibodies or immunoglobulins play one of the most important roles in immune response [4]. Antibodies react to specific antigens [1] — the alien substances which find their way into a body.

The immune response in an organism is caused by several of factors. So, to characterize the immune response one should investigate concentrations of these or those factors of immunity [2]. To select the effective personalized treatment, it is necessary to estimate also their activity, i.e. ability of antibodies to bind to antigens of the infectious agents which got to an organism. In modern medicine different optical methods are expected to be promising for therapy of infections and immune diseases [5, 6, 7].

In our work we used two spectroscopic techniques, laser correlation spectroscopy and fluorescence spectroscopy, for the immune system activity investigation. In previous work it was concluded that laser correlation spectroscopy allows estimation of the ability of biomolecules in biological fluids in dynamics [8]. Dynamic parameters are essential for immune studies because the reaction time shows the general immune activity. We decided to extend laser correlation spectroscopic experiments with fluorescence spectroscopic technique to get some new information about immune system such as conformational changes in immunoglobulins and other molecules in dynamics.
2. Materials and methods

2.1 Laser correlation spectroscopy
Laser correlation spectroscopy allows one to estimate sizes of nanoparticles in a fluid from measurements of scattered radiation parameters [9]. The experiments include recording of the time dependence of the scattered light intensity and the calculation of its autocorrelation function. According to the dynamic scattering theory [9], the autocorrelation function of the light scattered by particles in a solution will carry information on the diameters (D) of these particles.

To derive information about sizes of nanoparticles the distribution of scattered light intensity I(D) is counted. An algorithm based on Tikhonov regularization method and described in [10] is used for calculation of intensity distribution I(D) from autocorrelation function.

A typical scheme of the laser correlation spectroscopy device is shown in Fig. 1. In this setup, a laser beam is transmitted through a converging lens and focused on the cell containing the sample. Laser light is recorded at the angle $\theta = 90^\circ$ and passes through single mode optical fiber to a photomultiplier. The signal from the photomultiplier is digitized and arrives to a computer for the correlation analysis. More specific description can be found in [10, 11].

![Figure 1. Scheme of a laser correlation spectrometer. 1 — power supply for laser; 2 — laser radiation source; 3 — converging lens; 4 — sample; 5 — optical fiber; 6 — photomultiplier; 7 — analog-to-digital convertor; 8 — computer.](image)

2.2 Fluorescence spectroscopy
In fluorescence spectroscopy the fluorescence spectra and intensities are usually registered [12]. By measuring changes in the fluorescence intensity important conclusions about immune processes can be made, such as the general composition of antibodies, their conformational structure and activity [12]. A scheme of the experimental setup is shown in Fig. 2. Light from UV laser module passes through the attenuating filters, interacts with the sample deposited on the prism bottom causing its fluorescence. The fluorescence signal is detected by using a dynamic pin-diode [13], placed directly under the sample. Due to this design with a total internal reflection prism, the main beam does not pass to the pin diode and only the fluorescence signal intensity is measured. The output signal is analyzed on computer to calculate the fluorescence intensity [13].
2.3 Object of study
In this work mixed saliva (from all salivary glands) was chosen as the test biological solution. This biological material performs varied functions (cleansing, protective, immune, hormonal), because of its complex biochemical composition. It consists of proteins, lipids, carbohydrates, ions of sodium, potassium, vitamins et al. [14, 15]. Therefore, mixed saliva can be used for immune activity analysis. Promising new technologies have unveiled large numbers of medically valuable salivary biomarkers for different disease conditions including cancer, autoimmune, viral, bacterial, cardiovascular, and metabolic diseases [15 – 18]. Due to noninvasiveness of sample collection, smaller sample aliquots, cost effectiveness, easy storage and transportation, greater sensitivity, and correlation with levels in blood [19] mixed saliva has a great perspective in modern medical diagnostics [20, 21].

In our experiments the mixed saliva solutions with fluorescence spectrum in the range from 300 nm to 400 nm were used. A vaccine of tick-borne encephalitis containing antigens was used for triggering of immune response processes in saliva solutions. It was assumed that mixing saliva solutions with tick-borne encephalitis would provide binding of immunoglobulins with antigens in the vaccine.

Used chemical agents:
1. Aluminium hydroxide buffer;
2. Veronal buffer (VBS);
3. Natural saline solution;
4. Dried tick-borne encephalitis vaccine;
5. Saliva taken from healthy patient (women)

The mixed saliva was dissolved in VBS in 25% concentration. The tick-borne encephalitis vaccine was dissolved in aluminium hydroxide buffer with addition of natural saline solution.

3. Results and discussion
In the fluorescence measurements 0.02 ml of sample was placed on the glass under the prism. The fluorescence intensity was measured immediately after the samples preparation, after 45 minutes of incubation under the room temperature and after 2 hours of incubation. The measured fluorescence intensities are summarized in Tab. 1.

| Object                                      | Immediately after preparation | 45 min after preparation | 2 hours after preparation |
|---------------------------------------------|-------------------------------|--------------------------|----------------------------|
| Saliva dissolved in VBS with vaccine of tick-borne encephalitis | 14.29 mW/mm²                 | 1.06 mW/mm²              | 0.45 mW/mm²               |

Table 1. Calculated fluorescence intensities for mixed saliva with vaccine of tick-borne encephalitis addition
It can be noticed that the fluorescence intensity of saliva after addition of vaccine decreased while incubation. The decrease in fluorescence intensity can indicate reorganization of tryptophan – the fluorescent amino acid, part of almost all immune proteins [22]. The tryptophan fluorescence is higher when it is not blocked by other proteins parts. It was concluded that the fluorescence decrease suggests possible conformational changes in the structure of immune complexes and antigen antibody reactions. Saliva solution reacts with an antigen (vaccine of tick-borne encephalitis), blocking the tryptophan fluorescence, therefore the total fluorescence intensity decreases [22].

To confirm the existence of immune system reaction, measurements of particle sizes in saliva solution before and after the vaccine addition were taken. The results are presented in Fig. 3 – 6.

![Figure 3](image1.png)

**Figure 3.** Sizes of particles in saliva dissolved in VBS: 45 min after preparation.

![Figure 4](image2.png)

**Figure 4.** Sizes of particles in saliva dissolved in VBS: 120 min after preparation.
Figure 5. Sizes of particles in saliva dissolved in VBS with vaccine of tick-borne encephalitis addition: 45 min after preparation.

Figure 6. Sizes of particles in saliva dissolved in VBS with vaccine of tick-borne encephalitis addition: 120 min after preparation.

It can be observed that in the saliva solution there are no noticeable changes in particle sizes while incubation within 45 minutes – 2 hours. This demonstrates lack of reactions in the studied solution. However after addition of vaccine of tick-borne encephalitis (the measured particle size in pure vaccine equals to $5.8 \pm 0.4$ nm) in solution of saliva the sizes of particles are increased noticeably (minimal sizes increased from 100 nm to 150 nm) after 45 minutes of incubation and further incense (maximal sizes increased from 300 nm to 400 nm) after 2 hours of incubation. These results clearly demonstrate reaction of humoral immunity in human saliva on the antigen. By measuring sizes we could detect the degree of aggregation of immune complexes with antigens in vaccine: higher sizes, stronger reaction. In addition, we could measure time of immune reaction that shows how fast an organism can detect and incapacitate infections.
4. Conclusion
In this work the possibilities of spectroscopic techniques for medical diagnostics on the example of immune studies are demonstrated. By using the fluorescence spectroscopic technique we detected some conformational changes in saliva molecules showed by tryptophan fluorescence decreasing when the vaccine of tick-borne encephalitis was added. We suggested that the decrease in tryptophan fluorescence is a result of its blocking in the center of molecules and also about its aggregation. Laser correlation spectroscopic technique confirmed the aggregation theory and showed increasing aggregation sizes in time. By determining sizes and time of aggregation exploiting fluorescence measurements the immune response for each individual person could be measured. The further studies of immune system in human saliva and also in blood could open new perspectives for cheap diagnostics and individual therapy.

Acknowledgements
Authors are very grateful ActLight SA (Lausanne, Switzerland, http://act-light.com/technology) for their donation of pin-diode for that research. Also authors would like to thank O Tsybin and D Dyubo for great help in providing of fluorescence experiments.

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