

Investigation of Hair Microstructure Changes in Patients with Malignant Tumours by Optical Techniques

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Abstract. The study reports on the differences in hair microstructure of healthy people and patients with cancer. The research has experimentally proved that hair of healthy people has a dense structure with a prominent external layer. In contrast, the hair of cancer patients has a destroyed external layer that is partially absent due to the presence of ulcers. Hair structure defects relate to biochemical changes: cancer presence leads to the increased ratio between oxygen and nitrogen, primarily on account of the decreased nitrogen concentration and, consequently, the decreased peptide concentration. Peptides composition in hair structure was also investigated by fluorimetry. The spectra of fluorescence excitation have several maxima such as at 295, 350 nm. For cancer patients, the spectrum has a huge maximum at 295 nm, while the spectrum for healthy volunteers has a small maximum at the wavelength and is shifting toward shorter wavelengths. We assume that the peak at 350 nm is primarily defined by the elastin contained in hair. The maximum at 295 nm is connected with the presence of tryptophan-containing peptides presence, such as keratin extracted from the Henley layer with keratinized cells. The obtained results can be used to improve optical techniques of biomedical diagnostics of cancer diseases. © 2019 Journal of Biomedical Photonics & Engineering.

Keywords: cancer; hair; optical biomedical diagnostics; scanning electronic microscopy.

Paper #3339 received 5 Nov 2019; revised manuscript received 28 Dec 2019; accepted for publication 11 Dec 2019; published online 31 Dec 2019. [doi: 10.18287/JBPE19.05.040301](https://doi.org/10.18287/JBPE19.05.040301).

1 Introduction

One of the most important properties of modern techniques in clinical diagnostics is noninvasiveness. It is achieved by either improving the devices or by registration of biochemical properties of cells and tissues, which requires detailed investigation.

It is well-known [1] during tumour growth, the organ undergoes not only local changes. Cancer cells affect host metabolism through the circulating factors they secrete or induce the host to secrete [2]. This leads to disturbance of skin and its appendages [3]. Thus, hair and nails could serve as a health indicator for some

diseases that provoke changes in microelement distribution. Most of the published articles contain information about Ca [4], Mo [5], Fe [6] concentration and their selective accumulation in hair due to the presence of pathological processes. Therefore, the disease may be detected by the analysis of the microelement status of hair [7]. However, these technologies are not widely used because hair microelement status depends on many factors such as nutrition, diseases, shampoos, etc.

In this work, we demonstrate the differences in hair microstructure and their peptide contribution in healthy

volunteers and cancer patients for the purposes of improving optical biomedical diagnostics.

2 Materials and methods

2.1 Research objects

The object of this is hair of two groups of volunteers. The first group included 23 healthy women. The second group was composed of women with cancer disease of different localization admitted in the A. Tsyb Medical Radiological Research Centre and the Orenburg Regional Clinical Psychoneurological Hospital for War Veterans. Oncology was confirmed by blood and histological analysis carried out in hospital. Tumour localizations are listed in Table 1. The age of all women was 50 ± 20 years for cancer patients and 47 ± 18 years for healthy volunteers. All the patients signed the informed consent. The investigation was carried out in conformance with the Agreement of Ethical Committee of the Smolensk State Medical University.

Table 1 Distribution of the cases in the second group for investigation.

Diagnosis	Amount
Breast cancer	17
Colon cancer	3
Melanoma	2
Lung cancer	2
Stomach cancer	1
Pancreas cancer	1

2.2 Optical microscopy

After the hair aliquot has been cleared twice in medical alcohol, it was placed on the slide. The hair structure was investigated by means of an *Altami Inver 3* optical inverted microscope with a video camera working in the phase contrast mode (magnification 400 \times).

2.3 Scanning electronic microscopy (SEM)

The hair microstructure was investigated by means of a *JEOL-6000* scanning electronic microscope. Like for the optical microscopy, the cleared hair was placed on the slide. The measurements of microelements (nitrogen and oxygen) distribution and their quantitative analysis were carried out under high vacuum with voltage 20 kV.

2.4 Dynamic light scattering

The cleared hair aliquot was added to the free-prepared carbonate buffer (pH = 10) for 50 min. To measure the particle size, the obtained suspension was placed to *Photocor Compact*. The laser wavelength was 647 nm with the power of 25 mW. All the solutions used were

free-prepared from dry compounds produced by *Sigma-Aldrich*.

2.5 Spectrofluorimetry

The spectra of fluorescence excitation of solutions obtained as for dynamic light scattering were measured by the *SOLAR CM-2203* spectrofluorimeter. The wavelength of registration was 410 nm. The spectra were measured in the UV region.

3 Results and Discussion

Human hair is known [8] to have a layered structure. Therefore, any changes in metabolic processes lead to changes in the hair biochemical composition and to impact on the layer integrity. As a rule, its destruction starts from the external layer. The photos of the hair samples from healthy volunteers and cancer patients are presented in Fig. 1: the hair of healthy volunteers has a dense structure and a prominent external layer is prominent. In contrast, the hair of cancer patients is thinner with unclear structure. Fig. 1b and 1c depict possible defects of hair structure of cancer patients. Superficial defects in the external layer or its total focal absence are visible in the majority of samples from patients with cancer. The disturbed of geometry and absence of clear structure were observed in several specimens from cancer patients: the hair section has an oval form instead of the round form without scales. We noticed that hair colouring leads to insignificant changes of its structure. The cosmetic effect lasts for a short time (1–2 weeks) whereas the next hair generation gets the structure according to the physiological state of the body.

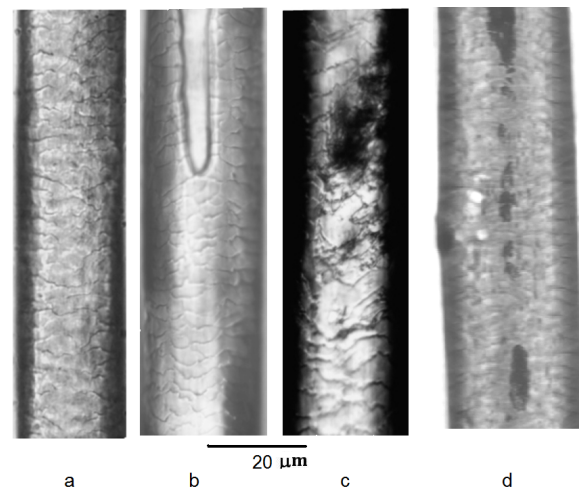


Fig. 1 The images of hair in healthy volunteers (a) and cancer patients (b, c, d) carried out by optical microscopy.

Changes in the hair structure are connected to biochemical changes. During the study, the concentrations of nitrogen and oxygen were registered. The results of the measurements are shown in Fig. 2.

These elements are basic in biological structures and present in all organic compounds. So, nitrogen is a component of amino acids, nucleotides and other vital substances. Concentration of oxygen, in turn, is primarily defined by reactive oxygen species dissolved in a cell and oxygen-containing organic substances.

As can be seen in Fig. 2, concentration of nitrogen decreases due to cancer. This dependence is observed in all samples and is well reproduced. Moreover, concentration of oxygen in the hair of healthy volunteers is lower than in the hair of cancer patients. Cancer cells are known [9] to actively consume peptides and molecular oxygen. As the result of cancer cells metabolism, reactive oxygen species are generated, which leads to cell destruction. Quantitative analysis of nitrogen and oxygen concentrations in the investigated groups depicted in Table 2 suggest that the overlap of weight percentage of oxygen and nitrogen is 100%, while the carbon concentration change is insignificant. The ratio $W(O_2)/W(N_2)$ is significantly different for these two groups and have statistically reliable means. The presence of malignant tumour leads to the increase of this ratio, on account of the decrease in nitrogen and the consequent decrease in peptide concentration. This dependence was demonstrated in hair samples in mice with spontaneous breast tumour [10].

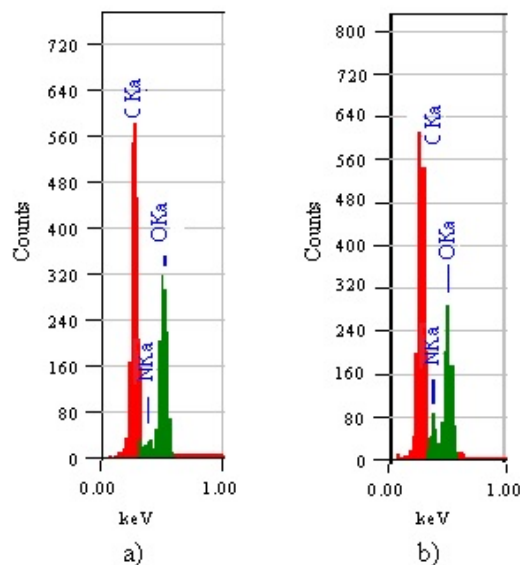


Fig. 2 Typical data of quantitative analysis of carbon, nitrogen and oxygen by SEM in external hair layer of cancer patients (a) and healthy (b) volunteers.

Table 2 The results of quantitative analysis of nitrogen and oxygen concentrations in the investigated groups.

	W(O ₂), %	W(N ₂), %	W(O ₂)/W(N ₂)
Health volunteers	78.9 ± 2.3	21.1 ± 1.3	3.7 ± 0.2
Cancer patients	75.2 ± 1.5	14.8 ± 1.1	5.1 ± 0.4

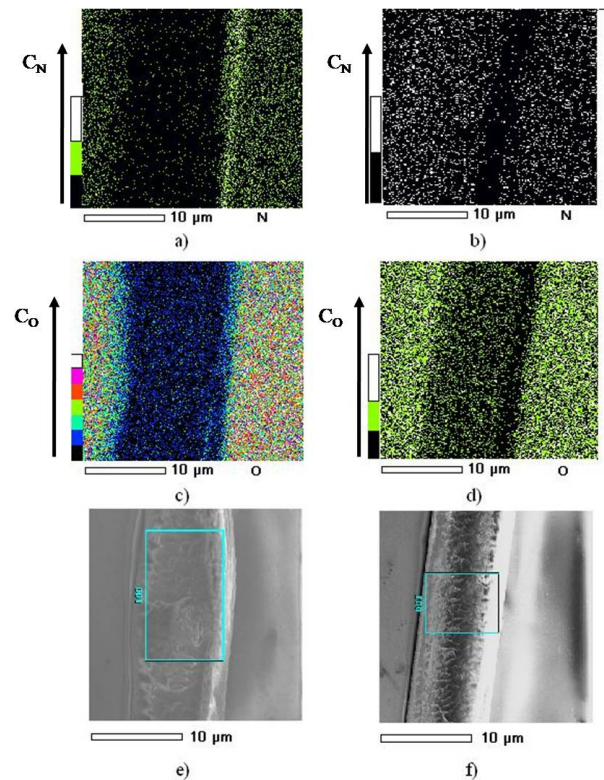


Fig. 3 Map of nitrogen and oxygen distribution in external hair layer of healthy volunteers (b, d, f) and cancer patients (a, c, e). The y-axis shows the colour of the picture with increased element concentration.

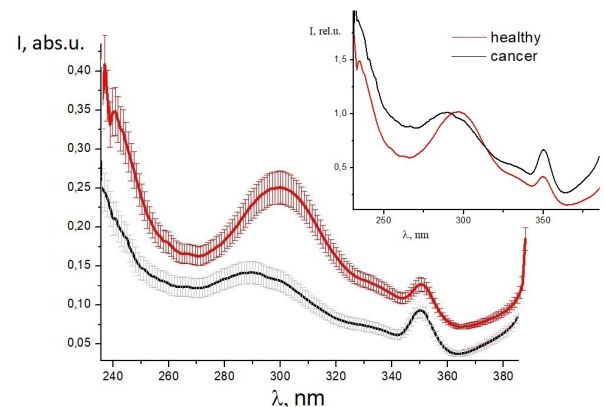


Fig. 4 The native (without processing) and normalized spectra of fluorescence excitation of extracted peptides from the external hair layer of healthy volunteers and cancer patients. The wavelength registration is 410 nm.

In cells, the distribution of the microelements is heterogeneous, which can be seen in Fig. 3. The white dots correspond to the highest concentration of the registered microelement, while the black dots correspond to nil concentration. The dark stripe in the centre of Fig. 3 shows that the concentration there is minimum. In our opinion, this may be attributed to the features of energy distribution of electrons scattering from the hair cylindrical surface. Each microelement has a range of reflected electron energy. Since the

reflected electron energy depends on the angle of scattering, the defined microelement concentration has its own spectrum and is heterogeneous through the entire hair surface. Thus, the microelement concentration should be compared with area with homogenous distribution (see the edge of the picture).

In healthy people, the distribution of oxygen and nitrogen is more homogenous (Fig. 3), but presence of malignant tumour leads to increased oxygen concentration, which may be due to increased concentration of reactive oxygen species. Change in nitrogen concentration for oncological patients has an adverse effect. It is known [9] that cancer cells assimilate hydrocarbons and peptides due to their own intensive metabolism. Nitrogen in cells transfers to other forms such as dioxide, ammonium ion, etc., which can be excreted from body. This leads to decreased nitrogen concentration. Moreover, as was proved in our previous work [10], data of external and internal layer can be experimentally observed due to the destruction of hair external layer in cancer patients.

The peptide impact on the results of SEM and their composition may be investigated by both laser flash photolysis and dynamic light scattering. However, we have found that hair has a strong intercellular matrix and, consequently, it is difficult to obtain cell suspension by hair fermentative treatment. Hence, peptides can be extracted from hair by treatment with carbonate buffer (pH = 10). As a result of the procedure, the peptides are leached. This method, described in the work of Fedorkova *et al.* [11], finally leads to the solution of the dissolved peptides in buffer. In the result of measurements by the technique of dynamic light scattering, we did not receive a significant difference in the size of the dissolved peptides. Two types of peptides were registered with an average radius for hair in healthy volunteers of 3451 ± 120 nm and 240 ± 62 nm. For cancer patients these means were 4132 ± 154 nm and 234 ± 58 nm.

However, structure of peptides can be detected not only by dynamic light scattering but also by fluorimetry. For this purpose, the spectra of fluorescence excitation could be measured (Fig. 4). The wavelength registration was selected taking into account the region of absorbance and fluorescence of the most common peptides. As can be seen in the Fig. 4, these spectra are different and have several maxima such as at 295,

350 nm. For the cancer patients, the spectrum has a huge maximum at 295 nm, but the spectrum for healthy volunteers has a small maximum at the wavelength and is shifting toward shorter wavelengths. We assume that the maximum at 350 nm is determined by the elastin contained in the hair [12]. The maximum at 295 nm is connected to the presence of tryptophan containing peptides [13], such as keratin [14]. It was found [8] that under the external layer of hair there is a Henley layer of keratin containing cells [15]. Since the disturbance of hair structure and the absence of the external layer are more expressed in the hair of cancer patients, the maximum at 295 nm is also more expressed for the hair of cancer patients.

4 Conclusion

Numerous investigations into hair structure and its element status touch provide data on peculiarities of hair for different nationalities [16] and age [5]. Our study revealed no significant difference in hair structure of people from different age groups. Moreover, the data about age-related changes in hair structure are controversial. In this respect, our data are close to the similar data obtained by another research team group [17] that showed insignificant age-related changes in hair structure for different age groups of the volunteers. Nonetheless, the possible age peculiarities are insignificant in comparison with the hair structure defects in cancer patients described in this work. The result of dynamic light scattering and fluorimetry demonstrate different peptides in hair structures of healthy volunteers and cancer patients. Registration of changes in peptides contribution could help to improve optical techniques of biomedical diagnostics of cancer.

Disclosures

All authors declare that there is no conflict of interests in this paper.

Acknowledgments

The authors greatly thank all the volunteers participating in the investigation and Ms. T. M. Tkacheva for their help in this work.

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