# Electrochemotherapy of skin cancer treatment results estimated by in vivo autofluorescence measurements

BILIANA NIKOLOVA[*], EKATERINA BORISOVA[a], EKATERINA PEYCHEVA[b], LATCHEZAR AVRAMOV[a], IANA TSONEVA

*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria, Acad. G. Bonchev str., Bl.21, 1110 Sofia, Bulgaria*

[a]*Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko chaussee Blvd., 1784 Sofia, Bulgaria*

[b]*Specialized Hospital for Active Treatment of Oncology, Plovdivsko pole 6 str. Sofia, Bulgaria*

Electrochemotherapy is an effective method for treatment of skin tumors. To monitor the effects of application of the electrochemotherapy autofluorescence spectra are taken from the lesion and surrounding healthy skin, prior to, immediately after treatment and at the control check-ups. Patients are followed up at the first week after treatment and one month later. Here we reported the case of 82 years old woman with basal cell carcinoma, which is treated with electrochemotherapy and the effect of the treatment is verified by the optical biopsy method.

(Received March 19, 2015; accepted June 9, 2016)

*Keywords:* Electrochemotherapy, Skin cancer, Basal cell carcinoma, Tumor autofluorescence

## 1. Introduction

After the first application of electrical pulses for effective introducing of anticancer drugs [1], electrochemotherapy became a powerful method for treatment of malignant cutaneous and subcutaneous tumors and gene delivery [2, 3]. The efficiency of electrochemotherapy of skin tumors is confirmed in several clinical trials [4, 5]. The procedure is based on electroporation (electropermeabilization) of the cell membrane that is associated with membrane disorder [6], change in electrical parameters [7] and the creation of aqueous pathways in the cell membrane as a result of application of short, intensive electric field [8]. This phenomenon speeds up the simultaneous administration of cytotoxic drugs, usually bleomycin or cisplatin. Backgrounds on the mechanism of electroporation, including analytic description of the transmembrane voltage induced in a single cell, in cell suspensions and in tissues, may be found in a monographic book chapter of Miclavcic and Kotnik [9].

Biomedical optics is one of the fastest growing areas of research. The possibility to apply light for investigation and detection of abnormalities in human tissues makes this technique perspective for the development of new diagnostic modalities.

Normal tissue autofluorescence spectrum, when excitation in the end of UV and blue spectral range is applied, consists mainly from fluorescence signals of collagen, elastin, protein cross-links, NADH and flavins [10, 11], with a maximum in the region of 450-520 nm. The autofluorescence spectra observed are distorted from the re-absorption of melanin in case of skin lesions and different forms of hemoglobin for skin and mucosa lesions respectively.

In the case of most common cutaneous tumor – basal cell carcinoma (BCC) usually the spectra obtained have not significant spectral shape changes vs. normal skin spectra, but are with lower intensity, which allow to reach sensible diagnostic accuracy. Advanced stages of BCC lesions reveal red fluorescence, which appear due to endogenous porphyrins, accumulated in the tumor areas [12].

The information we receive from the optical biopsy allow us to use it as a method for early cancer detection as well as a precise non-invasive tool for planning and monitoring of therapeutic procedures.

With this case report we presented the power of the combination of light-induced autofluorescence spectroscopy and electrochemotherapy as a new, non-invasive approach for successful monitoring and treatment of basal cell carcinoma.

## 2. Materials and methods

Electrochemotherapy currently is method with wide application at Dermatologic Clinic at Specialized Hospital for Active Treatment of Oncology, Sofia. The application and study of electrochemotherapy in patients was approved by the ethical committee of the hospital and all patients gave written informed consent before beginning treatment. Patients were cured according to the following criteria: clinically and cytologically confirmed basal cell carcinoma, age >18 years, appropriate for electrochemotherapy.

The patients were treated under local anesthesia (lidocain 1%). A pair of parallel flat electrodes with the adjustable inter electrode distance (caliper type) in the range 5-30 mm were used. The electrotreatment was done by 16 biphasic rectangular pulses each of them 50+50 μs duration with 20 μs interval between both phases and pause between bipolar pulses of 880 μs. The size of the lesion was measured by caliper instrument (Arimedex). Good contact between electrodes and the skin was ensured by a conductive gel. Electric pulses were delivered 10 min after intralesional injection of bleomycin. The treatment response has to be evaluated at least 4 weeks after the treatment according to WHO (Word Health Organization) guidelines as follows: 1) Complete response (CR): the absence of any trace of tumor; 2) Partial response (PR): decrease in the tumor volume by 50% or greater; 3) No response (NR): decrease of <50% or an increase of <25% in the tumor volume; 4) Progressive disease (PD): tumor volume enlarged more than 25%.

The patients were subject to the weekly examinations. To monitor non-invasively the effects of electrochemotherapy spectral analysis – light-induced autofluorescence spectroscopy was used. The method could be applied as a very precise tool for initial diagnostics, for planning and monitoring of therapeutic procedures.

For autofluorescence measurements of skin pathologies multiple wavelength excitation of the endogenous fluorescence of benign and malignant cutaneous lesions was applied. Initially, lesions were classified clinically by experienced dermatologist-oncologist. Second step was detection of lesion and surrounding normal skin autofluorescence using different excitation wavelengths, namely 365, 385, and 405 nm, received from several narrow-band light-emitting diodes. Optical fiber probe was used to deliver the light from LEDs and to collect the fluorescence signals from the skin surface. It consists of 7 fibers in circular geometry. Central fiber was used for autofluorescence signal detection and it was connected to microspectrometric system and surrounding six fibers were used for delivery of excitation light from the LEDs to the skin under investigation. Autofluorescence signals were recorded and stored using a fiber-optic microspectrometer (USB4000, Ocean Optics, Dunedin, FL, USA). A personal computer was used to control the system and to store and display the data using the specialized microspectrometer software OOI Base ("Ocean Optics", Inc., Dunedin, FL, USA). Normal tissue fluorescence was used as a basis for comparison with the pathologies observed. Three to five spectra were detected from one position and averaged to reduce the experimental noise of the spectrometer and inhomogeneity of the cutaneous area investigated for each excitation wavelength applied. Number of averages depends from the size of the lesions investigated.

Spectra were detected from the lesion and surrounding normal skin prior the electrochemotherapy procedure, immediately after the therapeutic procedure and at the control check-ups.

## 3. Case report

Here we reported a case of 82 years old woman Caucasian type with basal cell carcinoma, stage T1N0M0. The subject of the treatment is new onset lesion on the right cheek with dimensions 20x25.2x1 mm. Treatment strategy includes: an electric field – 1000 V applied to the longer side of the lesion (with intraelectrode distance of 30 mm, respectively electric field intensity 333 V/cm, amplitude) and 2 ml intralesional injection of the cytostatic drug bleomycin.

A

B

C

*Fig. 1. The status of the patient: A-before treatment, B - one week after, C - one month after*

The status of the patient was presented in Fig. 1 (A, B, C): A - before treatment, B - one week after, C - one month after.

To monitor the effects of application of the electrochemotherapy autofluorescence spectra are taken from the lesion and surrounding healthy skin, prior to, immediately after treatment and at the control check-ups. Patient was followed up at the first week after treatment, and one month later. Fig. 2 presented results from such therapeutic monitoring, using 365 nm excitation of BCC lesion and normal skin during the treatment procedure and on the first check-up. BCC tumor has lower intensity than normal tissue. It is clearly observed immediate reaction after therapeutic procedure application – appearance of specific minima at 543 and 575 nm, related to increased hemoglobin absorption. This increase of the local hemoglobin content is related to the reaction of the skin on electroporation process. One week later the fluorescence intensity of the lesion area is higher and approach to the "normal skin" spectral shape, which is indication for successful treatment of the tumor Fig. 2.

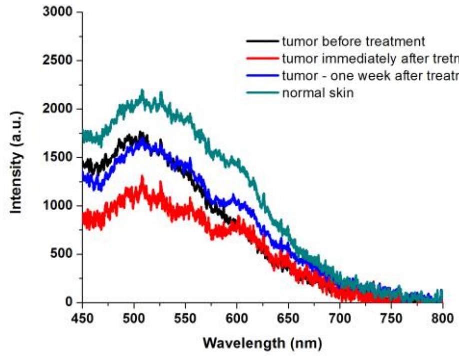


*Fig. 2. Comparison of autofluorescence spectra of normal skin and BCC lesion, before, immediately after electrochemotherapeutic treatment and a week later. Spectra are observed after 365 nm excitation*

On Fig. 3 are presented autofluorescence spectra for the same BCC lesion evolution before and after electrochemotherapy procedure, but using excitation at 405 nm. Here are presented the results before and after treatment procedure and for the initial two check-ups of the patient - on the seventh day and on the thirtieth day after the therapeutic intervention.

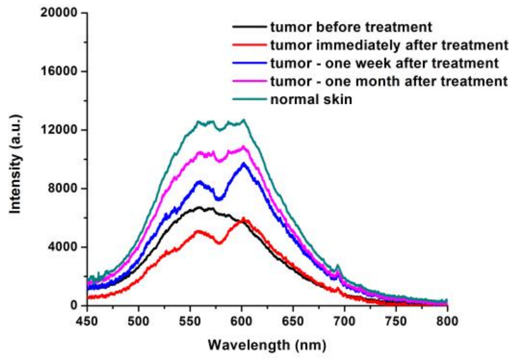


*Fig. 3. Comparison of autofluorescence spectra of normal skin and BCC lesion, before, immediately after electrochemotherapy and on 7 day and on 30 day of the patient' follow-up. Excitation wavelength applied is 405 nm*

Immediately after the electrochemotherapy procedure a rapid increase of hemoglobin content is observed in the fluorescence spectra for all excitation wavelengths applied – minima at 545 and 575 nm are well pronounced, see fig. 3. Relative increase of the emission peaks in the region of 560 and 610 nm is also observed, especially on the first week control measurements. The first maximum correlates to the increased level of flavins during the lesion healing process [10, 11, 13, 14]. Long wavelength fluorescence signal increase could be addressed to the appearance of endogenous porphyrins accumulated in the edema region during the process of lesion healing [11, 14] as well. However, no specific spectral signatures of protoporphyrin IX in the lesion investigated were observed.

## 4. Conclusion

Fluorescence spectra of normal skin, BCC lesion before and immediately after electrochemotherapy procedure, as well as one month follow up of the lesion, are presented. Clinical trial is currently under implementation and with broadening of the database with fluorescence spectra of major skin benign and malignant pathologies we expect to receive objective tool for cancer detection and treatment monitoring.

On the other hand the skin tumors are predominantly on the visible places of the body and to take biopsy after electrochemotherapy will give some scars. To monitor non-invasively the effects of electrochemotherapy spectral analysis–light induced autofluorescence spectroscopy is with a good cosmetically effect.

The combination of these two advanced methods (electrochemotherapy and light induced autofluorescence spectroscopy) could be applied as a very precise tool for initial diagnostics, for planning and monitoring of therapeutic procedures.

### Acknowledgements

This work is supported by DFNI-B02/9/12.12.2014 "Development of biophotonics methods as a basis of oncology theranostics". This work was conducted in the scope of the EU- COST Actions TD1104 and BM1205.

## References

[1] L. M. Mir, M. Belehradek, C. Domenge, S. Orlowski, B. Poddevin, J. Belehradek Jr, G. Schwaab, B. Luboinski, C. Paoletti, C. R. Acad. Sci., **313**, 613 (1991).
[2] B. Nikolova, E. Peycheva, Ts. Mudrov, T. Dobreva, M. Matveev, I. Tsoneva, Int. J. Bioautomation, **18**, 31 (2014).
[3] J. M. Escoffre, B. Nikolova, L. Mallet, J. Henri, C. Favard, M. Golzio, J. Teissié, I. Tsoneva, M. P. Rols, Cur. Gene Ther., **12**, 417 (2012).
[4] R. Heller, M. J. Jaroszeski, L. F. Glass, J. L. Messina, D. P. Rapaport, R. C. DeConti, N. Fenske, R. Gilbert, L. M. Mir, D. S. Reintgen, Cancer, **77**, 964 (1996).
[5] E. Peycheva, I. Daskalov, J. BUON, **4**, 185 (1999).
[6] J. M. Escoffre, E. Bellard, C. Faurie, S. Sébaï, M. Golzio, J. Teissié, M.-P. Rols, Biochim. Biophys. Acta, **1838,** 1701 (2014).

[7] M. Moisescu, M. Radu, E. Kovacs, L. M. Mir, T. Savopol, Biochim. Biophys. Acta, **1828,** 365 (2013).
[8] G. Saulis, R. Saule, Biochim. Biophys. **18**, 3032 (2012).
[9] D. Miclavcic, T. Kotnik, Medicine, Taylor and Francis (2004).
[10] Yu. Sinichkin, S. Utz, A. Mavliutov, H. Pilipenko, J. Biomed. Opt, **3**, 201 (1998).
[11] L. Bachmann, D. Zezell, A. da Costa Ribeiro, L. Gomes, A. Ito, Appl. Spectr. Rev., **41**, 575 (2006).
[12] E. Borisova, P. Pavlova, E. Pavlova, P. Troyanova, L. Avramov, Int. J. Bioautomation, **16**, 53 (2012).
[13] J. de Leeuw, N. Beek, W. Neugebauer, P. Bjerring, H. Neumann, Las. Surg. Med. **41**, 96 (2009).
[14] M. Skala, N. Ramanujam, Multiphoton Redox Ratio Imaging for Metabolic Monitoring in vivo, in Advanced Protocols in Oxidative Stress, Ed. Don Armstrong, 155-162, (2010).

* Corresponding author: nikolova@bio21.bas.bg