Image-guided Electro-assisted Drug Delivery: Comparison between Two Types of Electrodes

Biliana Nikolova[1*], Severina Atanasova[1], Tsvetan Mudrov[1], Iana Tsoneva[1], Zhivko Zhelev[1,2], Rumiana Bakalova[3,4], Ichio Aoki[3]

[1]*Institute of Biophysics and Biomedical Engineering*
Bulgarian Academy of Sciences
21 Acad. G. Bonchev Str., Sofia 1113, Bulgaria
E-mails: nikolova@bio21.bas.bg, severina@bio21.bas.bg, mudrovc@biomed.bas.bg, tsoneva@bio21.bas.bg, zh_zhelev@yahoo.com

[2]*Medical Faculty, Trakia University*
11 Armejska Str., Stara Zagora 6000, Bulgaria

[3]*Molecular Imaging Center, National Institute of Radiological Sciences*
4-9-1 Anagawa, Inage-ku
Chiba 263-8555, Japan
E-mail: bakalova@nirs.go.jp

[4]*Medical Faculty, Sofia University "St. Kliment Ohridski"*
1 Koziak Str., Sofia 1407, Bulgaria

[*]*Corresponding author*

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Abstract: *Electroporation-based cancer treatment techniques are currently after active investigations in the field of drug delivery, optimization of electrical parameters and elucidation of the exact mechanisms at a molecular level. The present study is designed to investigate the exact in vivo redistribution and persistence of nanoparticles in the tumor tissue of colon-cancer grafted mice after electroporation with two different kinds of electrodes. The aim of the study is to avoid artifacts during electroporation due to accumulation of nanoparticles in the surrounding non-cancer tissues. The isolated electrodes are appropriate for the treatment of 3-dimensional tumors and have a large potential in this field.*

Keywords: *Electroassisted drug delivery, in vivo imaging, Cancer, Nanoparticles, Quantum dots.*

Introduction

Electroporation is a biophysical phenomenon which is connected to the application of external electrical pulses across the cell membrane, aiming to increase its natural permeability [13, 16] Some drugs (e.g., bleomycin, cisplatin, nanoparticle-based, etc.), used in chemotherapy practices, have poor access to the tumor cells and electroporation offers a possibility for enhancing their local delivery [11, 15]. A lot of studies show several-fold potentiating of cytotoxicity of anticancer drugs after application of short high-voltage electrical pulses [4, 17]. The process is known as electrochemotherapy. Recently this method has been routinely used in oncological clinics [5, 14].

Electroporation-based cancer treatment techniques are currently after active investigations in the field of drug delivery, optimization of electrical parameters and elucidation of the exact mechanisms at a molecular level. Besides membrane electroporation, application of electrical pulses in tumor tissues causes blood flow reduction, thus inducing drug entrapment into

the target tissue for hours [18]. The exact distribution and retention of drugs into the tumor tissue are still disputable issues.

One appropriate approach for the elucidating of the above-mentioned questions is the development of highly target-specific and image-guided drug-delivery systems (DDS) with drug-release control, based on nanotechnologies [3]. Nanoscaled drug delivery systems (nano-DDS or nanoparticles) are constructed to deliver a high local concentration of drugs into the target locus (organs, tissues or even cells). The designing of nanocarriers, capable of selective disposition into the cancer cells and solid tumors, is an essential issue in the development of new diagnostic and therapeutic strategies in cancer [2, 9, 12]. The target-specific nano-DDS allow the achievement of a much higher local concentration of the encapsulated substances (drugs and/or contrast agents) in the region of interest, which can improve the diagnostic potential and therapeutic effect [6]. For target selective drug delivery and delivery of imaging probes polyioncomplex hollow vesicles (polymersomes) have been used as a rule for the last several years. Polymersomes are labeled with different contrast agents and their pharmacokinetics is verified *in vivo* by optical imaging, magnetic resonance imaging, positron emision tomography and multimodal imaging. Some of the most appropriate fluorescent markers for deep tissue optical imaging are the semiconductor quantum dots (QDs) due to their unique spectral properties [1, 3, 22]. In the last years there has been an increased number of publications about the application of facilitating and accelerating delivery techniques. Electro-assisted technics is also a promising tool.

The present study is designed to investigate the exact *in vivo* redistribution and persistence of nanoparticles in the tumor tissue of colon-cancer grafted mice after electroporation with two different kinds of electrodes. The aim of the study is to avoid artifacts during electroporation due to the accumulation of nanoparcticles in the surrounding non-cancer tissues.

Materials and methods

Chemicals

QD^{705} (Qdot®705 ITK^{TM} carboxyl quantum dots) were purchased from *Invitrogen*. Water-soluble polymersomes were prepared from chemically modified chitosan as described by Lee et al. [8]. Labeling of polymersomes with QDs was carried out via carbodiimide chemistry, using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) as a zero-length cross-linker [7]. The nanoparticles were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and fluorescent spectroscopy. QD concentration in polymersomes was calculated by the method of Yu et al. [21].

Isoflurane was purchased from *Abbott* (Japan). All chemicals used in this study were analytical or HPLC grade.

Experimental cancer model

Balb6 nude mice (21 ± 2 g) were used. *Conol 26* cells ($1x10^5$ in 10 μL PBS, pH 7.4) were inoculated subdermally in the left/right hindpaw. All measurements were performed ~ 9-10 days after inoculation, when the tumor size was ~ 100 mm^3.

All experiments were conducted in accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of the National Institute of Radiological Sciences, Chiba, Japan.

Optical imaging

All experiments *in vivo* were conducted under anesthesia. Briefly, the mouse was anesthetized with 1.5% isoflurane using a mask. The tail vein was catheterized for administration of nanoparticles and the mouse was fixed in the camera of the *Maestro EX Imaging System*, connected to the anesthesia device. The body autofluorescence was registered at excitation filter 435-480 nm and emission filter 700 nm longpass. Nanoparticles (QD^{705}-labeled polymersomes) were injected intravenously (i.v.) via the tail vein (single dose – 80 nmol; 100 μL volume) and the whole body fluorescence was registered on the back side at different time-intervals.

The data were analyzed by *Living Image In Vivo Imaging* software (*Maestro* version 2.10.0).

Electroporation

An electroporator "Chemopulse IV", generating bipolar pulses, was used in the experiments [5]. The instrument was equipped with a large voltage control in the limits of 100-2200 V, simplified operations, locking against illegal manipulations and enhanced protection against electrical hazards. The electro-treatment was carried out by 16 biphasic pulses, each of them of 50+50 μs duration with a 20 μs pause between both phases and a pause between bipolar pulses of 880 μs.

Two types of parallel stainless steel electrodes (caliper type) with adjustable intra-electrode distance in the range of 1-30 mm were used, the first one without a bottom insulator and another one with a 1.5 mm insulator to minimize the side-effects of electroporation (e.g., accumulation of nanoparticles, drugs or contrast substances outside the tumor area) (Fig. 1). Electrical pulses with intensity of 1000 V/cm were applied.

Fig. 1 Two types of electrodes used in the present study: (A) New electrode with bottom isolation (left part); (B) Standard electrode without isolation (right part)

Results and discussion

Aiming to study the effect of electroporation on drug and/or nanoparticles *in vivo* distribution in tumor-grafted mice models, we apply electrical pulses after intravenous (i.v.) injection of QD^{705} labeled nanosomes. The images, obtained 2 min after injection (without and followed by electroporation with a standard electrode), are presented in Fig. 2. In both cases, the tumor was visualized in this short time-interval due to angiogenesis. Three hours after treatment, the fluorescent intensity was comparatively high in the tumor area of the electroporated mice (Fig. 2E), while without electroporation the fluorescent signal disappeared (Fig. 2B). In the case of electroporation, the fluorescence intensity is higher

due to penetration of the nanoparticles in cancer tissue and trapping into the tumor area. The traces of standard electrodes are detected in non-cancer tissue near the tumor (Fig. 2E). Presumably, a part of QD-labeled polymersomes are arrested outside the tumor tissue – in the skin covering the implanted tumor. Thus a part of nanoparticles are lost. Figs. 2C and 2F present *in vivo* fluorescence intensity 24 hours after i.v. injection with or without electroporation using standard electrode. A significant difference between electroporated and non-electroporated mice is observed. Weak tumor fluorescence with clearly visible traces of electrodes is shown in Fig. 2F, and there is no fluorescence in the absence of electroporation (Fig. 2C).

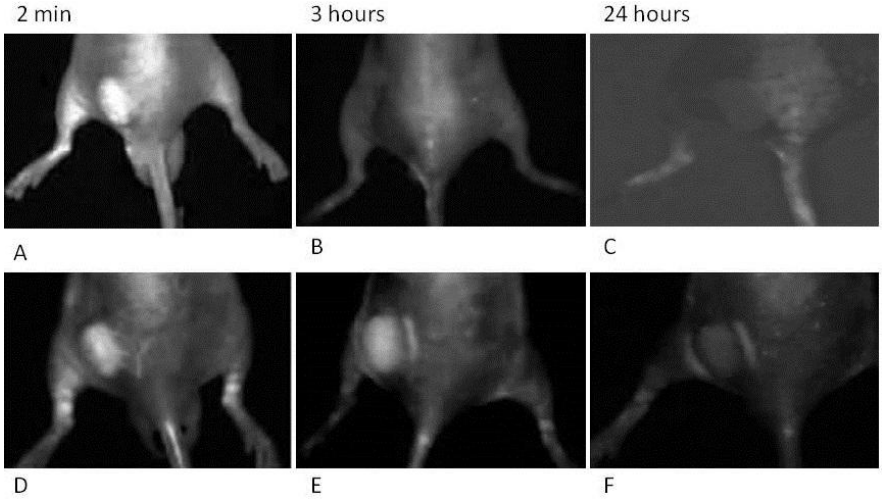

Fig. 2 Images of colon cancer-grafted mice obtained: 2 min, 3 hours, and 24 hours after i.v. injection of polymersomes without electroporation (A-C) and 2 min, 3 hours, and 24 hours after i.v. injection of polymersomes with electroporation using standard electrode (D-F)

To avoid side effects of electrotreatment of tumor-grafted mice and to enrich the concentration of nanoparticles inside the tumor area, we decided to isolate the bottom of the electrodes with the epoxy resin insulator. Fig. 3 shows the fluorescence intensity after electroporation using new isolated electrodes. Traces of electrodes are not detected near the tumor area. In the tumor area, the fluorescence intensity is comparatively high even 24 hours after electrotreatment (Fig. 3E).

Currently, the electroassisted therapeutic strategies in cancer are under active investigation. Several studies about the dependence of current density and electric field spatial distribution [10] on the geometry and position of used electrodes have been published [10, 19].

The influence of the material of the electrodes is also a disputable issue [20]. To our knowledge, there are no data about the exact distribution of the drug applied during electrochemotherapy.

Our study shows that the redistribution of the QD^{705} labeled polymersomes (or drugs) into the tumor area after electroporation depends on the type of electrodes. In our particular case, the isolation of electrodes in the bottom side enhances the delivery of nanoparticles into the tumor tissue without "losing material" in the surrounding area. The nanoparticles are trapped inside the tumor within 24 hours after combined treatment with nanoparticles and electroporation.

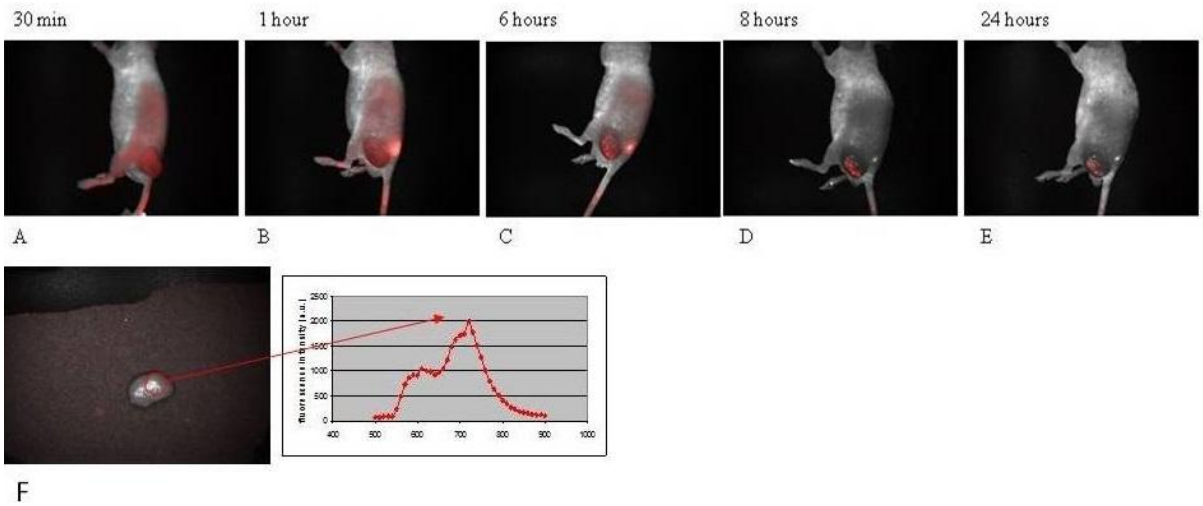

Fig. 3 Images of colon cancer-grafted mice obtained: 30 min (A), 1 hour (B), 6 hours (C), 8 hours (D), 24 hours (E), and image and fluorescence intensity spectra of *ex vivo* tumor 24 hours (F) after i.v. injection of polymersomes and electroporation using new resin isolated electrodes

From our clinical practice we can present a few cases of patients with Carcinoma basocellulare and hyper pigmentation on the sides of electrode application even one month after electrochemotherapy using standard (non-isolated) electrodes (Fig. 4).

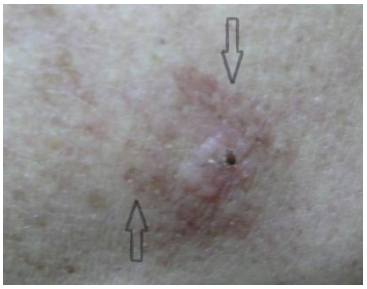

Fig. 4 Effect of electrochemotherapy (using standard electrodes) with bleomycin of a patient with Carcinoma basocellulare; the arrows point to the traces of the electrodes

Despite the presented side effect of electrochemotherapy, the application of the bottom resin isolated electrodes in the cure of human skin tumor lesions is ineffective due to the fact that most of them are flat (usually they are not three-dimensional).

The isolated electrodes are appropriate for the treatment of 3D tumors and have a large potential in this field.

Conclusion

In this study, we present *in vivo* fluorescence imaging of the distribution of QD^{705}-labeled polymersomes into the tumor area of colon-cancer grafted mice after electrotreatment with two different types of electrodes. The results demonstrate that better coverage of the tumor is essential for the effectiveness of the electrochemotherapy of three-dimensional tumors.

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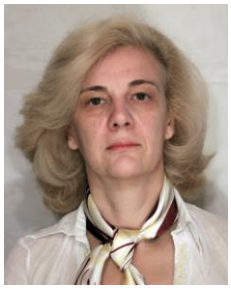
Assoc. Prof. Biliana Nikolova, Ph.D.
E-mail: nikolova@bio21.bas.bg



Biliana Nikolova graduated from Sofia University "St. Kl. Ohridski", Faculty of Biology, with a specialization in Biochemistry and Microbiology in 1992. In 1992 she started working in the Institute of Biophysics, BAS. She received her Ph.D. degree in 2001 in the same institute. Since 2013 she has been an Associate Professor in the Institute of Biophysics and Biomedical Engineering (IBPhBME). Her scientific interests are in the field of electroporation, electroloading, drug delivery systems, in vivo imaging and electrochemotherapy.

Severina Atanasova, Ph.D. Student
E-mail: severina@bio21.bas.bg

Severina Atanasova graduated from the Sofia University "St. Kl. Ohridski", Faculty of Biology, B.Sc. in Molecular Biology in 2011; M.Sc. in Biophysics in 2013. She received her Postgraduate Qualification "Teacher in Biology" in 2013. Since January 2014, she has been a full time Ph.D. student in the IBPhBME, BAS. Her scientific interests are in anticancer drugs, electrochemotherapy, fluorescent imaging, nanoparticles and drug delivery.

Tsvetan Mudrov, Ph.D.
E-mail: mudrovc@biomed.bas.bg

Tsvetan Mudrov graduated from the Technical University – Sofia, Faculty of Electronic Engineering and Technology, with a specialization in Electronic Medical Equipment in 2001. Since 2005, he has been working in Schiller Engineering. He received his Ph.D. degree in 2012 in the IBPhBME, BAS. His scientific achievements are focused on the development of high voltage defibrillator solutions, embedded programming in the field of biomedical signals processing for ECG instrumentation and defibrillation.

Prof. Iana Tsoneva, D.Sc.
E-mail: tsoneva@bio21.bas.bg

Iana Tsoneva received her Ph.D. degree in 1976, became an Associate Professor in 1990, a D.Sc. in 2005 and has been a Professor since 2006. She was the head of the Department of Electroinduced and Adhesive properties at the IBPhBME, BAS from 1994 to 2014. Her interests are in the field of electroporation, influence of the electrical field on the membrane permeability, electrotransfer of macromolecules in cancer and normal cells, electrical properties of biological membranes, electrochemotherapy, electroinduced changes in cell adhesion – adhesion, cell proliferation, viability in normal and neoplastic cells. Prof. Tsoneva is the author of more than 70 papers in the field of electroinduced effect.

Assoc. Prof. Zhivko Zhelev, Ph.D.
E-mail: zh_zhelev@yahoo.com

Zhivko Zhelev graduated from the Sofia University "St. Kl. Ohridski", Faculty of Biology, with a specialization in Biochemistry in 1988. He has worked in the BAS (as a researcher), the National Institute of Advanced Industrial Science and Technology of Japan and the National Institute of Radiological Sciences of Japan (as a senior researcher), the Medical Faculty of Trakia University and the IBPhBME of the BAS (as an Associate Professor). He received his Ph.D. degree in 2010 in the Medical Faculty of Trakia University. His scientific interests are in the field of molecular imaging, molecular probes, nanomedicine, drug delivery systems, oxidative stress, electrochemotherapy, etc.

Prof. Rumiana Bakalova, D.Sc.
E-mail: bakalova@nirs.go.jp

Rumiana Bakalova graduated from the Sofia University "St. Kl. Ohridski", Faculty of Biology, with a specialization in Biochemistry in 1986. She has worked in the BAS (as a researcher), the Medical University-Sofia (as an Assistant Professor), the National Institute of Advanced Industrial Science and Technology of Japan, the National Institute of Radiological Sciences of Japan (as a senior researcher), and the Medical Faculty of the Sofia University (as a Professor). She received her Ph.D. degree in 1990 in the BAS and a D.Sc. degree in 2011 in the Sofia University "St. Kl. Ohridski". Her scientific interests are in the field of molecular imaging, molecular probes, nanomedicine, drug delivery systems, oxidative stress, electrochemotherapy, etc.