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TOPICAL PROBLEMS OF BIOPHOTONICS

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Invited

LASERS IN CARDIAC SURGERY: FOURTEEN YEARS EXPERIENCE

I.I. Berishvili, L.E. Chvichia, P.V. Gusev, M.S. Semenov, and **Yu.V. Ignatyeva**

Bakoulev Cardiovascular Scientific Center, RAMS, Moscow, Russian Federation, tmlr@rambler.ru

Objective

Transmyocardial laser revascularization (TMR) has emerged as an alternative therapeutic option for patients with severe diffuse coronary artery disease refractory to conventional modes of therapy. However, only in patients treated with CO_2 laser results are explained by improvement of perfusion.

We present results of 692 TMR operations performed with different lasers.

Methods

More than 750 patients underwent TMR from April 1997 to April 2011. Isolated TMR was performed in 243 patients, 443 patients underwent combined CABG with TMR. In 152 of these cases TMR was used in combination with CABG on a beating heart. In 642 cases TMR was performed with high power CO_2 laser, 30 with XeCl laser, and 22 with low energy laser «LASON».

Results

Hospital mortality was 1.7%, for the last 500 operations there was only 2 hospital deaths (0.4%) and 7 late deaths. Overall mortality rate was 2.8%. Mortality rate after operations with CO_2 laser was 1.5%, and after low power lasers 17%.

Postoperative tallium scan controls (SPECT) after lasing with CO_2 laser demonstrated significant impovement in stress-induced ischemia in the majority of patients. PET study revealed restoration of segments with hibernating myocardium. No such improvement was seen in cases with XeCl and «LASON» lasers. Comparative assessment of the available rates of postoperative congestive heart failure, myocardial infarction, and arrhythmias demonstrated a higher rate of all of these complications for patients treated with low energy lasers. Therefore, the real improvement of patients' condition with amelioration of perfusion and normalization of myocardial metabolism is seen only with the use of CO_2 laser. The procedure with CO_2 laser is safe and provides a long-standing effect. With growing experience, hospital mortality is low and mortality and major adverse events are minimal in the follow-up for up to 10 years.

The beneficial effect after TMR performed with low power lasers in the 1-year after TMR was lost in 36 months. Mortality and morbidity in the follow-up are high. What is the explanation for these differences?

Obviously, it is related to incorrect definition of the goals. For transmyocardiac revascularization needles and other devices were used in the first studies. But results fueled the hypothesis of direct perfusion channels occluded in postoperative period. This leads to the use of laser irradiation, that would cause channels endothelization and, subsequently, improve patency. In reality they will be closed, but for many years the investigators studied the hypothesis of channel patency. It was the first false assumption. The next stage was dedicated to search of laser devices, forming smooth-walled channels. Then came search of lasers causing minimal lesions. All this led to implementation of various lasers, including those that were unsuitable for TMLR.

In this connection one should probably emphasize that the primary goal of TMR is not a creation of the channels. The primary goal is in formation of thermal injury for the initiation of angiogenesis. This thermal necrosis is nothing more than tubular burn scars. It is well known that size-limited scars will induce angiogenesis due to resulting local hypoxia and the involved neutrophyles releasing number of angiogenic factors.

Conclusions

The distinction in wavelengths of light between low energy and CO_2 lasers have increasing importance in the results. Operations performed with low energy lasers demonstrated significant mortality and morbility. TMR created with CO_2 laser is safe and effective procedure. At 7-year follow –up in patients with end-stage CAD that precluded conventional modes of therapy TMR showed significant functional improvement as well as improvement of the quality of life.

Invited

IN VIVO AUTOFLUORESCENCE SPECTROSCOPY FOR CLINICAL APPLICATION IN ONCOLOGY

N.N. Bulgakova[1], V.V. Sokolov[2], and **K.A. Vereschagin[1]**

[1]A.M. Prokhorov General Physics Institute R A S, Moscow, Russia; nnbulgakova@gmail.com
[2] P.A. Hertzen Moscow Research Oncological Institute, Moscow, Russia

Over the last 10 years, autofluorescence spectroscopy and imaging have been widely studied to improve the detection of early stage cancer by many scientific groups [1, 2]. As the malignant process advances and epithelium status transits from normal to dysplastic one and then to CIS and invasive cancer, alterations can be found in the tissue architectonics and spatial distribution of endogenous chromophors, as well as in the concentration and metabolic activity of certain endogenous chromophors and fluorophors. These alterations translate into the intensity and spectral pattern of autofluorescence emission from the tissues, resulting in a gradual decrease in the autofluorescence intensity in visible spectrum at the sites of neoplastic alterations [1]. As a result, small malignant lesions can be visualized as darkened sites against the background of intense green autofluorescence emission of normal tissue. Several models of autofluorescence endoscopes are available now for autofluorescence diagnosis (AFD) of bronchial early stage cancer and stomach cancer. However, a subjective nature of the visual assessment of autofluorescence images and lack of quantitative information on the intensity and spectral composition of fluorescence restrain the potentials of the method. Thus, autofluorescence images provide low specificity in detection of early recurrent or residual tumors after previous treatments or thoracic surgery [1, 3].

Local fluorescence spectroscopy (LFS) is a sensitive method that makes it possible to measure tissue fluorescence spectra using fiber-optic probe scanning in contact with the tissue surface. The main advantage of LFS is noninvasive real-time collecting of quantitative information on the intensity and spectral features of the tissue fluorescence emission. A combination of fluorescence imaging with *in vivo* LFS improved quality and reliability of the photodynamic diagnosis of bladder and lung cancer [3, 4].

In order to improve the potentials and specificity of AFD of early stage lung cancer, a large sample of laser induced autofluorescence spectra of bronchial epithelium with different morphological status (normal mucosa, inflammation, dysplasia and early stage cancer) have been collected. A complex of main spectral parameters (9) of autofluorescence emission spectra recorded in vivo under 532 nm laser excitations were studied. The spectral parameters that characterize some features of the intensity and shape of autofluorescence spectra for reliable differentiation between healthy and malignant bronchial epithelium have been revealed. Monitoring of these parameters in the course of autofluorescence bronchoscopy examination could be helpful for minimizing the number of biopsies necessary as well as detection of early malignancy. Also, the results obtained allow concluding that AFD is feasible for detection of the early recurrent malignant lesions in the bronchial stump 6 months after thoracic surgery.

Acknowledgements

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Invited

EXPERIMENTAL JUSTIFICATION OF LASER PUNCTURE TREATMENT OF SPINAL OSTEOCHONDROSIS

V.M. Chudnovskii[1], **V.I. Yusupov**[1,2], and **V.N. Bagratashvili**[2]

[1] Il'ichev Pacific Institute of Oceanology, Far East Division, Russian Academy of Sciences
Baltiiskaya ul. 43, Vladivostok, Russia
[2] Institute on Laser and Information Technologies, Russian Academy of Sciences
Pionerskaya ul. 2, Troitsk, Moscow Region, Russia, bagrat@laser.ru

Spinal osteochondrosis is the most widespread neurological disease of adults in economically developed countries. The most abundant and serious manifestation of spinal osteochondrosis is hernia of spinal disc, which results in development of compressive pain syndrome, motive dysfunctions of lower limbs and other serious complications. Surgery is requires to about 20% of such patients.

One of the tendencies in the treatment of spinal osteochondrosis is application of medium-power (1–5 W) radiation. Such approaches are aimed at substituting laser nucleothomy, the technology which is based on total laser ablation of nucleous pulposus of disc and gives complications. The modern treatment of spinal osteochondrosis is based on the formation of multiple channels in the intervertebral disk using a silica fiber with the carbon-coated silica fiber tip which is heated by laser radiation with a wavelength of 970 nm and a power of 3 W [1–2]. It is commonly accepted that the surgical destruction of lesion focus and the recovery of tissue in all the above procedures result from the laser destruction and heating of tissues, which are provided by the local heating of the distal fragment of the optical fiber and the effective absorption of laser radiation in water and tissues. However, in many cases, the developed therapeutic effect related to the moderate-power laser irradiation cannot be interpreted using thermal effects only, since the majority of organs with connective tissues (e.g., disks and bones) are not heated by laser irradiation [1–2]. It is clear that thermal destruction is insufficient to explain a sharp decrease in the density of herniation immediately after laser manipulation which results in the transformation of hernia into a soft sponge, since the herniation is relatively far from the region of the channel formation.

For experimental justification of such medium-power laser treatment technique, we have studied acoustic response originated from spinal disc in the course of laser treatment of spinal disc *in vivo*, and, also, in the case of *in vitro* laser irradiation. It is shown that laser irradiation of ware-saturated tissue may result in generation of intense shock-type waves. Such effects are caused by the contact of laser overheated (up to 700 – 1000ºC) fiber tip with water-saturated cartilage tissues of spinal discs and explosive boiling of water solution and burning of collagen (and other components) in cartilage. Generation of multiple gas-water bubbles alters pressure inside the irradiated disc up to $5\ 10^3 – 10^4$ Pa and higher. The pressure rise is modulated by low-frequency (~ 1 – 10 Hz) vibrations, and, also by (60 – 150 Hz) shock-wave type vibrations with amplitude ~ $10^2 – 3\ 10^2$ Pa and even higher. We believe that these low-frequency vibrations are caused by periodic depressurization, which takes place because of water vapor release from laser irradiation zone, while shock-wave type vibrations are originated from degenerative spinal disc with hernia, which serves as a Helmholtz-type resonator.

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Invited

PRELIMINARY EXPERIENCE OF AUTOFLUORESCENCE MAMMARY DUCTOSCOPY

A. Douplik[1,2], W.L. Leong[2], A.M. Easson[2], S. Done[2], G. Netchev[2], and **B.C. Wilson[2]**

[1]Erlangen University, Germany
[2]Ontario Cancer Institute, Canada
alexandre.douplik@aot.uni-erlangen.de

Mammary ductoscopy (MD) has been used as a diagnostic tool for ductal carcinoma, which is the most common form of cancer in the breast [1]. Here, we sought to enhance the diagnostic accuracy of MD by the addition of autofluorescence imaging [2] and diffuse reflectance and fluorescence point spectroscopy [3]. We assessed whether there are distinct changes in the tissue autofluorescence images between malignant and benign tissues that potentially can facilitate visualization of lesions that are not seen under conventional white light ductoscopy. For simplicity, we used an existing autofluorescence imaging system that had been developed and optimized for GI endoscopy [4]. A priori, this is not necessarily optimal for ductoscopy, so that we included the point fluorescence and reflectance spectroscopies to gain further data that may inform the interpretation of the imaging results and, potentially, to provide additional information that may be used to optimize the imaging parameters for this specific application. Ten consented patients with a pre-operative diagnosis of palpable invasive ductal carcinoma who required a mastectomy were recruited into the study between May 2005 and July 2008. This *ex vivo* pilot study was approved by Research Ethics Board at University Health Network, Toronto. All mastectomy specimens were examined within 1–1.5 h after resection. Two different ductoscopes were used. The first 3 cases were carried out with a 1.1 mm external diameter instrument. However, this was found to limit access, so that the remaining 7 cases were done using a 0.7 mm ductoscope. Both devices had a working length of 70 mm, field of view of 70º ± 5º and depth of view of 1–10 mm (rigid fiber ductoscope, model MS-611, Fibertech, Japan). The ductoscope was coupled via a standard eyepiece to a fluorescence endoscopic imaging system (OncoLIFE, Xillix Technologies Corp, Canada: now PINPOINT, Novadaq Technologies Corp, Canada). Figure 1 shows examples of ductoscopic images, in both white-light and autofluorescence modes, from a 53 year old woman who underwent a modified radical mastectomy for a 3.5 cm invasive ductal carcinoma (AJCC T2N1M0). As shown in Fig 1, the images had adequate brightness and resolution in both imaging modes. Figures 1a and 1b show images taken from a normal duct from the same patient in white-light and fluorescence modes, respectively. In white-light mode, the intraductal carcinoma appeared as irregular protrusions into the ductal lumen, with color similar to the surrounding ductal tissue (Figure 1c). In fluorescence mode (Figure 1d), the same intraductal carcinoma appeared reddish compared to the surrounding ductal tissue, which was blue-green (cyan) in color. It was noted that debris seen in the lumen of the normal duct (Figure 1a), which can be misinterpreted as cancer, appropriately appeared blue-green in fluorescence mode, demonstrating the ability of fluorescence ductoscopy to differentiate cancer from non-cancer tissue in this case.

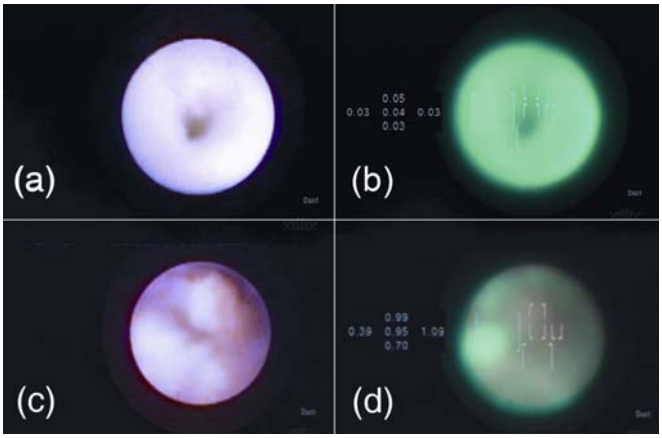

Fig 1. Normal region ((a) white light, (b) autofluorescence) compared to tumor region ((c) white light, (d) autofluorescence). The forward looking field-of-view view is approximately 1 mm (Modified from [3])

Figure 2 shows an example of the reflectance and autofluorescence spectra.

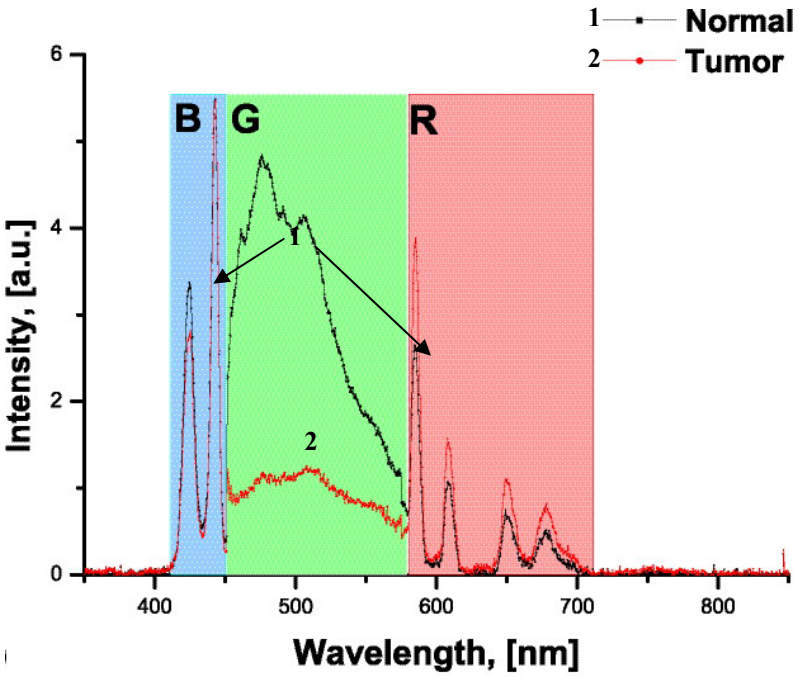

Fig. 2. Autofluorescence spectra in the green band (G) providing also acquisition of some portions of blue (B-band) and red (R-band) reflectance from the excitation light. The sharp peaks within the blue (B) and red (R) bands are mercury lines from the arc lamp source (Modified from [3])

The point spectroscopy and imaging gave qualitatively similar results. Since ductoscopy can access ducts about 6-10cm from the nipple openings, our judgement is that >95% of breast cancers and papilloma with nipple discharge can be seen.

The next step will be to carry out similar studies in vivo and in patients who do not have known malignancy. We also plan to apply hyperspectral imaging system as a versatile tool to discover new algorithms for cancer identification in the future.

Acknowledgements

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Invited

APPLIED BIOPHOTONICS IN PRE-CLINICAL RESEARCH AND ITS FURTHER PERSPECTIVES FOR CLINICAL PRACTICE

Ch. Dullin

Dept of Diagnostic Radiology
christian.dullin@googlemail.com

Optical imaging technologies are increasingly in high demand to investigate the occurrence and location of molecular events as well as to follow target-specific probes in vivo. Especially, near-infrared (NIR) fluorescent probes enable observation of molecular events in deeper tissue. Here, we will present the use of NIRF imaging to optimize pre-clinically the concept for selective tumor therapies. A four laser based time-domain fluorescence imager, the Optix MX2, was applied to characterize binding specificity and kinetics of carrier molecules such as NIR fluorescent labeled monoclonal antibodies, antibody fragments and peptides to tumor cells *in vivo* in various mouse tumor models.

The visualization of the expression and activity of a trypsin-like transmembrane serine protease by NIRF imaging to evaluate a matriptase-based therapy pre-clinically in an orthotopic pancreatic tumor model will be presented. By applying optical imaging, fluorescently labeled endothelial progenitor cells could be traced at the site of injury in intimal lesions and fluorescent labeled natural killer cells were detected within tumors. Furthermore, we will present evidence that lifetime-based *in vivo* fluorescence imaging is a powerful tool to depict specific probe-derived fluorescence signals. Fusion of NIRF imaging data with high-resolution volume computer tomography *in vivo* is shown in order to correlate biological processes with anatomical sites.

Moreover, the potential of applied biophotonics in clinical application will be presented. Here, most promising tools are endoscopic fluorescence-based devices as well as breast and skin imaging in combination with NIRF labeled target probes for example to detect early tumors as well as to monitor tumor progression in response to therapy. A further application is intraoperative scanning that facilitates detection of residual tumor tissue at the resection edge to assist the surgeon and even the pathologist during oncologic surgery. These clinical application for optical imaging are less limited by the low penetration depth providing effective and low-invasive procedures for future translation of these technologies in patient care.

Invited

RADICAL AND PALLIATIVE PDT PROGRAMS IN COMBINED TREATMENT OF MALIGNANT NEOPLASMS

M. Gelfond, A. Arseniev, G. Gafton, V. Klimenko, V. Gelfond, and **V. Egorenkov**

N.N. Petrov Scientific Research Institute of Oncology, St.Petersburg, Russia
mark.gelfond@gmail.com

Intraoperative PDT is used in the N.N. Petrov Institute of Oncology for treating malignant pleurites in the case of primary and secondary pleura tumors during video thoracoscopy (V.T.S.), and also for stomach cancer surgery.

Materials and techniques

V.T.S. for treating chemoresistant forms of exudative pleurites was used for 176 patients.

Surgical treatment of stomach cancer with intraoperative PDT was applied to 34 patients aiming at cytoreduction of possible tumoral cells remaining after lymphodissection (D2) and improvement of remote results of treatment.

Photosensitiser Fotoditasin was administered at the beginning of narcosis injection in a conventional medical dose of 50mg. PDT was performed with Atkus-2 laser (Joint-Stock Company «Semiconductor Devices», St.-Petersburg). In the case of pleural tumors the entire pleural cavity was exposed to irradiation. Combined pleurodesis was performed at intensive, more than 500ml per day exudations.

In the case of stomach carcinoma surgery, intraoperative PDT is performed in areas of anastomoses, and also in zones of localization of lymphatic collectors after lymph dissection irradiation.

Results

Termination of liquid accumulation, improvement of external breath function, indicators of blood gas parameters, rising of Karnoffsky index from 60 to 70 % were noted within 6 months in all the patients who had undergone pleurodesis. Contrary to prognosis, full clinical remission of patients was observed in 11 months on the average (from 6 to 29 months). Partial clinical remission was attained in 5 months on the average (from 3 to 10 months). 50% of patients survived more than 1 year after pleurodesis, 13 % in 2 years; 37 % of patients died within 1 year (primarily, 7-10 months after operation). Full cupping of painful syndrome and reduction of a tumoral intoxication became was observed in all patients. Comparison of various pleurodesis methods showed that the combined method is more effective (by 25 %) than talcous pleurodesis.

Results of pilot studies of intraoperative PDT on 34 stomach carcinoma patients with IIIb tumoral process were analyzed. The minimum term of observation was 2 years. According to dynamic observation (computer tomography and ultrasonic investigation), there were no signs of recurrence and metastases in 87% of patients. In the control group the two-year survival rate was 11 %.

Conclusion

Thus, application of intraoperative PDT is an effective component of the combined treatment of malignant neoplasms. So, the palliative program of PDT in the treatment of stomach cancer and secondary exudative pleurites, especially its chemoresistant forms, is the effective method of palliative treatment, essentially improving the quality and in some cases the survival time of patients.

Invited

IN VIVO IMAGING OF ORAL MUCOSA MICROSTRUCTURE BY OPTICAL COHERENCE TOMOGRAPHY FOR MUCOSITIS SEVERITY PREDICTION

A. Maslennikova[1,2], N. Gladkova[3], I. Balalaeva[2,3], E. Kiseleva[1], M. Karabut[1,2], and **R. Iksanov [3]**

[1] Institute of Applied Physics RAS, Nizhny Novgorod, Russia, orlova@ufp.appl.sci-nnov.ru
[2] N.I. Lobachevsky State University of Nizhny Novgorod, Russia
[3] Nizhny Novgorod State Medical Academy, Russia

Mucositis is the most common oral complication of non-surgical therapy (chemotherapy, radiotherapy, and radio(chemo)therapy) in patients with head and neck cancer. Currently there are no in vivo methods to visualize microscopic changes of mucosal structure during a course of radio(chemo)therapy and to assess the development and healing of oral mucositis. As a result it has been impossible to predict its severity before evident mucosal changes occurred. The objective of this study was to visualize and quantify changes of normal oral mucosa in patients with oral and oropharyngeal cancer during a course of radio(chemo)therapy using a new non-invasive optical imaging modality – optical coherence tomography (OCT), which creates real-time cross-sectional microstructural images of tissues at depths of up to 2mm and with a spatial resolution of 10–15 mm.

Eighteen patients with stage II–IV of oral and oropharyngeal squamous histologically proved squamous cell carcinoma (SCC) of the oral cavity (7 patients) and oropharynx (11 patients), without distant metastases were enrolled in the study. The patients had no history of prior malignant neoplasm and had not received prior chemo- and/or radiotherapy. Treatment was planned according to the localization and extent of the tumor. Irradiation was performed using a Cobalt-60 unit or for external beam radiotherapy. Seven patients were treated with a split course of standard fractionated radiotherapy up to a total dose of 66–70 Gy; eight patients received a split course of radiotherapy up to the same dose, combined with concurrent chemotherapy (5-fluorouracil+cisplatin). Three patients received a course of preoperative radiotherapy up to a total dose of 44–46 Gy with standard fractionation. OCT imaging was performed daily starting on the first day of treatment at two points on the right and left cheek. A time domain OCT system operating at 1310 nm wavelength creating real-time cross-sectional images of tissues at depths of up to 2 mm with a spatial resolution of 10–15 μm was used. Dynamics of epithelium thickness and contrast level between epithelium and submucosa (evaluated as a tangent of inclination of approximating curve) was measured in the OCT images and compared with clinical manifestations.

OCT images of normal buccal mucosa had a high-contrast stratified structure with well-delineated epithelium, lamina propria, and submucosa. Their typical changes during radio(chemo)therapy included a gradual loss of contrast between the layers, signs of edema, and epithelium thinning, eventually leading to its complete absence (erosion). Shortly after the beginning of radiotherapy, the contrast between the epithelium and underlying lamina propria was reduced in all OCT images (Figs. 1a–c). When the total dose had accumulated to 12–16 Gy, clinical signs of mucosal changes in response to the irradiation, such as hyperemia and edema, became evident (Fig. 1c). As for all other investigated patients, the OCT image taken on the day of the clinical manifestation of mucositis had a substantially reduced contrast between the epithelium and the lamina propria (Figs. 1c,d) or even complete loss of these layers (Fig. 1e).

Substantial differences of mucosal microstructure dynamics were revealed in patients with grade III and grade II mucositis. The average decrease rate of the contrast between epithelium and submucosa was significantly higher in case of severe mucositis comparing to mild mucositis (0.68+0.04 Decibel/Gy vs 0.24+0.03 Decibel/Gy, $p = 0.003$).While epithelium and connective tissue can be identified in the images, the epithelium thickness decreased with the same rate regardless of mucositis severity. The average decrease rate was 5.1+1.4 μm/Gy and 4.4+0.8 μm/Gy in the case of grade II and grade III mucositis, respectively, without any differences between the groups ($p = 0.943$). Based on these results, exactly the contrast was chosen as a predicting criterion of mucositis severity (Fig. 2). Patients with complete loss of OCT contrast between epithelium and lamina propria on the first day of clinical manifestation ultimately had severe mucositis.

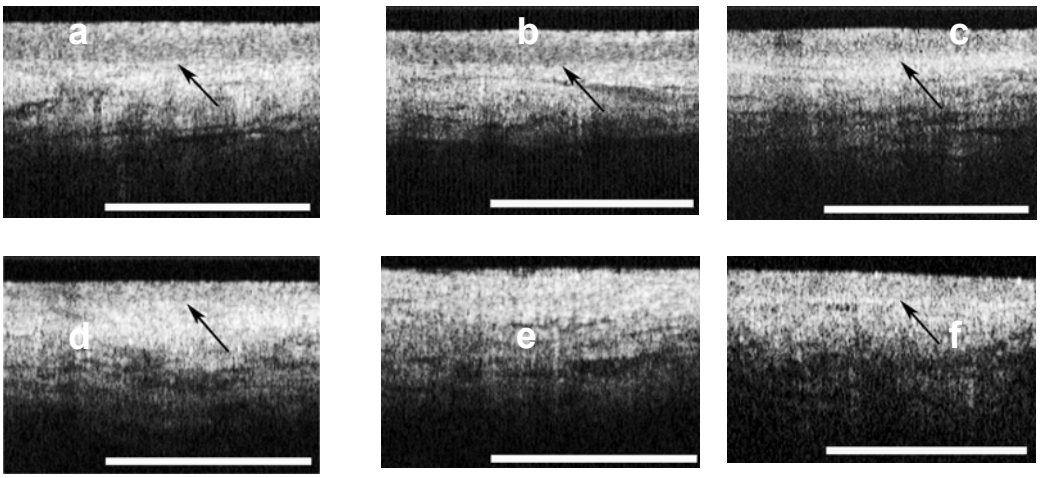

Fig. 1. Alteration of OCT images of buccal mucosa during a course of radiotherapy; 51-year-old male patient with cancer of oral cavity, stage II, with grade 2 mucositis. (a) Before irradiation; (b) after a total dose of 4Gy with no clinical changes, but reduced epithelial thickness in OCT; (c) after a total dose of 12 Gy; (d) after a total dose of 18 Gy. White scale bars measure 1 mm. Interface between the layers is shown by an arrow when visible

Patients who had a visible interface between epithelium and lamina propria on the first day of clinical symptoms had mild mucositis then.

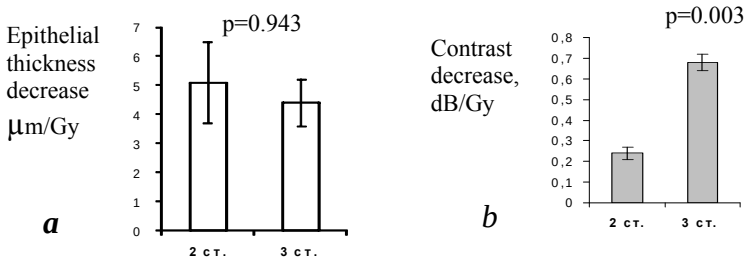

Fig. 2. Speed of epithelial thickness decrease (a) and contrast of OCT image decrease (b).

OCT provides additional valuable information about the state of mucosa in a course of radio(chemo)therapy to complement visual evaluation. Quantitative analysis of contrast between tissue layers in OCT images provides an objective tool to assess mucosal damage and repair during and after the treatment, allowing predicting the severity of radiation induced mucositis.

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Invited

APPLICATIONS OF ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY IN DERMATOLOGY

M.C. Meinke[1], **A. Lauer**[1], **B. Taskoparan**[1], **S. Haag**[1,3], **N. Groth**[2], **R. Bittl**[3], **A. Fahr**[4], and **J. Lademann**[1]

[1] Charité – Universitätsmedizin Berlin, Department of Dermatology, Berlin, Germany
martina.meinke@charite.de
[2] Privatinstitut Galenus GmbH, Berlin, Germany
[3] Freie Universität Berlin, Department of Physics, Berlin, Germany
[4] Friedrich-Schiller-Universität Jena, Department of Pharmaceutical Technology, Jena, Germany

Introduction

The skin is our barrier against the environment and protects us from pollutants, radiation exposure, etc. Both solar radiation and other stress factors induce free radicals in the skin. As a defense against these radicals the skin has developed an antioxidant protective system, which can be supported by systemic and topical application of antioxidants. Furthermore, the skin acts as a barrier for topically applied substances and it is a major skill to optimize formulations or carrier systems to transport active substances into the viable epidermis. The major obstacle for skin penetration of drugs is the superficial horny layer of the skin, which only allows limited penetration of small molecules with amphiphilic properties. ESR spectroscopic methods are presented, which permit analysis of systemically and topically applied antioxidants and monitoring of penetration enhancement and stabilization of active compounds or drug analogues.

Antioxidative capacity of the skin

The uptake of antioxidants with food supplements is, although very popular, controversially discussed. Regarding the uptake of products to enhance the radical protection in the skin, it must be clarified to what extent the substances are available in the skin and become effective there. In addition to Resonance Raman Spectroscopy, which is capable of determining carotenoids in the skin non-invasively [1, 2], EPR spectroscopy was used for the first time for the detection of the antioxidative capacity [3]. After the application of a permitted spin probe (stable test radical) to the human skin in vivo, various reductants besides carotenoids are involved in the reduction of the spin probe, which can be considered as an objective but not as a universal indicator of the antioxidative capacity of the skin. In two groups of volunteers, the reduction of a test radical in the skin was measured before and after the uptake of carotenoid-free antioxidants (Aronia) and a placebo product, respectively, after which the rate constant was determined. The verum group showed higher decay rates after the uptake than before the uptake, whereas no changes were detected in the placebo group. Furthermore, no change in carotenoid concentration could be observed.

Oxidative stress analysis induced by radiation

Whereas conventional sunscreens provide protection in the UV range, recent studies have shown that radicals can also be formed in the visible (VIS) and infrared (IR) spectral ranges. Since people are frequently tempted by the use of sunscreens to stay in the sun for an extended period, that is to say when sunscreens are misused, the formation of radicals by VIS/IR irradiation is currently in the focus of scientific investigations [4, 5]. Using ESR spectroscopy it is possible to determine the radicals in the skin and thus the antioxidant protective function of topically applied sunscreens in the IR spectral range. After application of various commercially available sunscreens to the skin, the radical formation in the skin after IR irradiation was measured in vitro. To explain the different protective functions, the antioxidant and optical properties of the creams were analyzed. As a result, it was found that both high scattering properties and high antioxidant capacities of the creams led to a reduced radical formation in the skin after exposure to IR irradiation [6].

Monitoring of penetration and stabilization effects of nanocarriers

Various transport systems are used in pharmaceutics and cosmetics for penetration enhancement, stabilization and enhanced storage of drugs applied to the skin. Invasomes – consisting of phosphatidylcholine, ethanol and terpenes – were shown to be effective drug delivery systems, but no

EPR-based data are available in the literature regarding penetration enhancement and enhanced storage.

Therefore, the partitioning of the hydrophilic spin label PCA (3-carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxy) and the amphiphilic TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) between membrane and aqueous phase has been studied by multi-frequency EPR. Furthermore, label partitioning and penetration were monitored during invasome penetration into porcine skin using EPR based methods. Subsequently, formulations containing invasomes and spin labels were applied to the forearms of human volunteers and penetration enhancement and stabilization were measured. Invasomes were able to enhance the penetration of the hydrophilic spin label PCA and could stabilize the reactive label TEMPO in vitro, as well as in vivo. The partitioning of the label TEMPO between membrane and aqueous surrounding could be monitored during penetration into the skin [7].

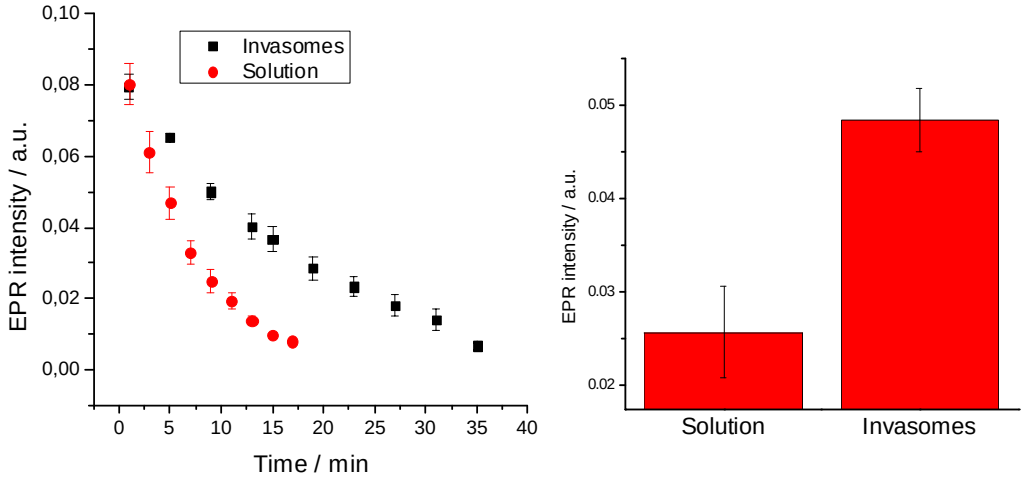

Fig. 1. Stabilization of TEMPO (left) in the skin and penetration enhancement of PCA (right) by invasomes compared to free label in solution

Hence, it could be demonstrated that ESR spectroscopy is suitable for determining the antioxidant capacity in vivo, following the systemic application of antioxidants and the protective function of topically applied substances in the skin in vitro. Furthermore, EPR can be used to monitor partitioning of a label within environments differing in polarity, as well as enhanced storage and penetration enhancement of spin labels into the skin.

Acknowledgements

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Invited

LASERS IN THE TREATMENT OF BENIGN PROSTATIC HYPERPLASIA: CONCEPTS AND CLINICAL RESULTS

R. Muschter

Department of Urology, Diakoniekrankenhaus Academic Teaching Hospital
Rotenburg, Germany
muschter@diako-online.de

Since 1990, different laser systems and laser-based treatment concepts for the treatment of benign prostatic hyperplasia (BPH) have been used. Employing the Nd:YAG laser, initial procedures were transurethral laser-induced prostatectomy (TULIP), visual laser ablation of the prostate (VLAP) and interstitial laser coagulation (ILC). Despite the challenging terminology, their common principle was coagulation, which was adhered to a common problem: the requirement of time for sloughing or resorption of necrotized tissue after an initial phase of edema of the prostate, leading to the need for postinterventional catheterization for days or weeks and delayed success. Therefore, at an early stage in the evolution of laser procedures, laser vaporization was introduced, still using the Nd:YAG laser, later laser resection and enucleation, respectively. The new laser for these latter procedures (HoLRP and HoLEP) was the Ho:YAG laser, which was until then mainly used for orthopedic surgery. Laser vaporization remained in its infancy for some years, because newly introduced electrosurgical devices had proven to generate similar tissue effects but much cheaper. The second era of laser vaporization began with a laser system emitting visible green light with hemoglobin being the defined target for absorption.

Currently, proven and accepted concepts of laser treatment for BPH are laser vaporization and laser enucleation. Both concepts are named after the predominant effect. Looking closer at the effects of laser-tissue-interaction, there is always some wanted or unwanted vaporization and coagulation if thermal energy is produced. These side-effects are of different quantity depending on the laser wavelength, laser power or pulse energy, respectively, and other parameters. Several lasers have proven their general capability to be used for prostatic tissue vaporization or enucleation, these are the greenlight lasers of different generations, diode lasers of different wavelengths and wavelength combinations, different thulium-based lasers and the Ho:YAG laser.

The desired clinical effects of laser treatment in general are tissue ablation and hemostasis. To compare currently employed commercial laser systems, only few experimental studies are available. Comparing clinical studies and their outcomes, however, it became obvious that vaporization is easier to learn but limited to smaller size prostates. Transurethral enucleation is a technique at first uncommon to urological surgeons and requires a longer learning curve, it is only useful if combined with tissue morcellation, but can be done safely in any size prostate. Although case-control studies (CCTs) and case reports exist for most lasers and techniques demonstrating the feasibility of treatment of high risk patients or patients using anticoagulative medication, this was addressed and proven in particular in larger CCTs only using the green light laser.

A large number of CCTs, some reporting long term follow-up of several years, and randomised controlled studies (RCTs) using lasers for BPH, have been published during the last decades. With the exception of HoLEP, which is the far best investigated procedure, the systems currently used are lacking RCTs, and long-term follow-up CCTs. According to RCTs comparing HoLEP to transurethral resection of the prostate (TURP) and open enucleation, clinical improvement of symptoms, quality of life, and voiding parameters, are comparable, while severe complications are reduced, and catheter and hospitalization times shortened. Risk reduction seems to be also the most important advantage of other laser systems compared to TURP, and in short-term CCTs clinical results are convincing.

Lasers did become important tools in the current treatment strategy of BPH. TURP, however, was still not replaced completely, and the ’laser battle’ was still not won by any single system.

OCT IN REPRODUCTIVE GYNECOLOGY

O. Panteleeva[1], N. Shakhova[2,3], G. Gelikonov[2], and **E. Yunusova[3]**

[1]Clinical Hospital of the Russian Railways, Nizhny Novgorod, Russia, o.g.panteleeva@yandex.ru
[2] Institute of Applied Physics RAS, Nizhny Novgorod, Russia
[3] Nizhny Novgorod Medical Academy, Russia

One of the principal factors of female infertility is tubo-peritoneal, with pathogenesis of pelvic inflammatory diseases (PID) and endometriosis. Besides infertility, these diseases rank as the 2-nd and 3rd most frequent causes of chronic pelvic pain (CPP) syndrome [1, 2]. In 45% of cases infertility and pelvis pains are caused by the so-called "microscopic forms" of endometriosis (endometrioid heterotopia is not more than 0.5 cm in diameter) [3–5], which require verification by laparoscopy and biopsy. Unfortunately, the cause of infertility is not clear in all cases. On the one hand, it points to a great number of asymptomatic and subclinical forms of PID and endometriosis. Subclinical inflammation accounts for 60% of all PID cases [6]. On the other hand, it is a consequence of the imperfection of the diagnostic capabilities of current methods. Optical coherence tomography (OCT), an imaging modality characterized by high spatial resolution, noninvasiveness, and high rate of image acquisition, is very promising for using in reproductive medicine [7].

Material and methods

194 female patients were enrolled in the study. The inclusion criteria were the following: reproductive age (18–45) and indications for laparoscopy, such as infertility, endometriosis, PID, and the CPP syndrome. All the patients signed the informed written consent.

OCT imaging was done during standard laparoscopy using a high-speed “OCT-1300U” device. It enables OCT imaging in the video-frame mode in real time and assessing morphofunctional features of the studied tissue. OCT device specifications are following: probing radiation wavelength 1280 nm, in-depth resolution 15 μm, lateral resolution 30 μm, imaging time 8-10 frames per second, probing depth up to 2 mm, replaceable endoscopic probe 2.7 mm in diameter.

The capabilities of OCT for diagnosing subacute inflammations and endometriosis were studied in two stages. At the first stage, the main task was to compare OCT images and data of histology for developing OCT criteria of the studied pathology. For this we acquired OCT images of unaltered organs and pelvic tissues: parietal peritoneum, ligaments, and fallopian tubes. The next step was OCT imaging of the pathology under consideration. Towards this end, OCT studies were carried out on PID patients with planned removal of fallopian tubes as a preparatory stage to supplementary reproductive technologies, and with endometriosis subject to laparoscopy and biopsy. In this fashion we have obtained typical OCT images of tissue in norm and pathology verified morphologically, which enabled us to develop OCT criteria of the studied pathology for comparative analysis of OCT and histological data (54 cases).

At the next step (OCT studies on 140 patients), we used the developed criteria to assess their diagnostic efficacy in blind recognition with subsequent statistical evaluation of results of the tests.

OCT images of endometriosis and PID

Our study demonstrates that there are a number of typical OCT patterns corresponding to structural alterations, which are caused by PID and "microscopic forms" of endometriosis.

For OCT diagnosis of endometriosis we studied pelvic peritoneum and pelvic ligaments. OCT studies of zones suspicious for endometriosis gave typical images: irregularly shaped optical hypointense inhomogeneity with contrast hyperintense contours and optical inhomogeneity of internal elements. The depth of such formations location relative to tissue surface is variable. Because of the small size (1–2 mm) of these areas and their localization under the parietal periotoneum it is impossible to visualize them during laparascopy. Results of histological analysis confirmed the presence of endometriosis.

One of the key objects of reproductive gynecology are fallopian tubes, morphological and/or functional alterations of which are the most frequent causes of female infertility. The current prevalence of latent forms of inflammation hinders diagnosis of such changes. We used OCT to study internal structure of the muscular membrane of fallopian tubes. First of all we acquired OCT images of

different parts of unaltered fallopian tubes in female patients of different age groups and at different phases of menstrual cycle. OCT images of the infundibulum and ampulla of uterine tube were greatly variable depending on age and phase of menstrual cycle, which makes these images unfit for reference. Stable optical characteristics were obtained for the isthmus of uterine tube. The OCT image of the wall of unaltered fallopian tube in this part is structureless, with moderate signal level and gradually decreasing signal intensity from the top towards the image bottom. The upper border of the image is even, there is actually no lower border, the rate of signal intensity decrease is uniform.

Two types of OCT images of fallopian tubes were obtained for the case of PID. The first of them are inhomogeneous images with dominating transverse striations and alternating zones of low and high signal level. Zones with low signal level may be rather large, of variable size, irregular contours, round or oval in shape. In the second type of images zones with high signal level prevail. Comparative analysis of OCT and histology data shows that images of the first type correspond to subacute inflammation accompanied by edema, and images of the second type to chronic inflammation with fibrosis.

At the final stage of the research we have done a blind test on recognition of inflammatory changes in fallopian tubes by means of OCT. Ten respondents were enrolled, 470 recognitions were made. Results of the tests demonstrated that the sensitivity of OCT diagnostics is 91.6%, specificity 78.4%; and diagnostic accuracy 78.9%.

Conclusion

We suppose that such imaging technique as OCT may be a valuable diagnostic tool for subclinical PID. The obtained data confirm that OCT is capable of detecting signs of inflammation to a high probability (higher than 90%) and excluding inflammatory changes to a rather high probability (higher than 75%). OCT is also an appropriate method for detection of small forms of endometriosis. In clinical practice it should be used as a complementary method with standard laparoscopy. For PID it demonstrates high diagnostic accuracy level; it can be used for recognition of inflammatory alterations and specific form of inflammation. In the case of endometriosis it can be used as a substitution of conventional biopsy when morphological verification is required.

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Invited

NEAR-INFRARED IMAGING OF BREAST CANCER USING OPTICAL CONTRAST AGENTS

A. Poellinger[1], C. Schmitz[2], P. Schneider[1], and **D. Grosenick[3]**

[1] Charité, Department of Radiology, Berlin, Germany, alexander.poellinger@charite.de,
[2] NIRx Medizintechnik, Berlin, Germany, [3] Physikalisch Technische Bundesanstalt, Berlin, Germany

Introduction

Breast cancer is the most common malignancy in women worldwide and the second leading cause of cancer death [1]. Routine screening reduces mortality from breast cancer, and X-ray mammography remains the key method for breast cancer screening with an average sensitivity of 75% [2]. However, mammography performs substantially worse in women with radiographically dense breasts and sensitivity often does not reach more than 30%-40% [3]. The limitations of available diagnostic techniques have led to an intensified search for alternative techniques in recent years.

Near-infrared (NIR) diffuse optical imaging is one of several new techniques with potential application in radiology that are currently under development for detection and characterization of breast lesions. While established modalities mainly rely on morphologic information, optical imaging has the potential to provide information on the molecular and cellular level that may improve detection and characterization of tumorous tissue [4, 5].

Material and Methods

For the last six years we performed several studies on NIR-imaging using three different scanner designs and two fluorescent dyes.

A) CTLM and Omocianine: A modified Computed Tomography Laser Mammography (CTLM, IDSI, Fort Lauderdale, Fl, USA) employs two rows of 84 collimated photodiodes and a continuous wave (CW) laser source arranged in a ring that surrounds the breast. A laser wavelength of 745 nm is used as it is near the Omocianine excitation peak at 761 nm. There is no direct contact between fibers and skin. In a multicenter study, 52 patients were examined with dose-escalating injections of Omocianine, a new fluorochrome with high quantum yield.

B) NIRx and indocyanine green (ICG): The DYNOT 232 optical tomography system (NIRx Medical Technologies, Berlin, Germany) consists of 31 fiber optic sensors, each containing an optical source and detector that are placed in contact with the breast. The instrument performs sequential illumination at each fiber position while simultaneously acquiring detector readings for each illumination site, thus acquiring 961 measurements at a rate of approx. 2 Hz. The scanner performs simultaneous dual-wavelength measurements at 760 nm and 830 nm. Thirty patients were scanned before and after administration of 25 mg ICG.

C) PTB (Physikalisch Technische Bundesanstalt, Berlin, Germany) optical mammograph and ICG: The prototype scanning optical mammograph records projection mammograms in transmission at several laser wavelengths and within a selected fluorescence band [6]. The breast is slightly compressed between two parallel glass plates. A 780 nm picosecond laser excites ICG fluorescence in the breast. The breast is scanned by synchronously moving the source fiber and the detection fiber bundle at a step size of 2.5 mm, and optical mammograms are recorded within 5 to 10 minutes.

Results

CTLM and Omocianine: There were 22 benign and 31 malignant lesions revealed by histology. An overall detection rate of 11.8% for benign and 44.4% for malignant lesions was found in the absorption mode. In the fluorescence mode, the detection rate reached 17.6% for benign and 55.6% for malignant lesions across all dose groups. Best sensitivity was obtained for dose group 0.1 mg/kg with a detection rate of 100% for malignant lesions in the fluorescence mode and 71.4% in the absorption mode. Lesion detection of Omocianine-enhanced fluorescence DOT showed dependence on the lesion-skin distance (< 20 mm: 63.6%, < 30 mm: 47.4%, ≥ 30 mm: 25%) and lesion size (< 20 mm: 53.8%; ≥ 20 mm: 61.5%).

NIRx and ICG: For 25 analyzed breasts peak-time amplitude (PTA) based analysis allowed correct detection in 12 of 14 malignant cases (sensitivity = 85.7%). False negative detection was observed for

a 15 mm invasive ductal carcinoma and a mixed intralobular ductal carcinoma (16 mm). Of 11 benign cases, one fibroadenoma (10 mm) was falsely detected (specificity = 91.0%). Peak-time amplitudes differed significantly between the benign and malignant group (Mann-Whitney U test, $p < 0.05$). Malignant lesions showed higher peaks at early time points in ICG perfusion. Eight patients had to be excluded due to insufficient breast coupling, large breast size, and different histological findings (T-cell lymphoma, melanoma metastasis). Thirteen of the 15 malignant lesions (86.7%) were found to be correctly localized. In four breasts (five lesions) additional spots were seen in false locations (33.3%).

PTB and ICG: All 13 carcinomas were visible in the absorption-corrected fluorescence ratio images, which featured high contrast between tumors and surrounding breast tissue with a contrast of 25% to 64% (figure 1). One of the eight benign lesions showed enhanced contrast in fluorescence scans during the late phase of ICG, and all three fibroadenomas were not visible. Visibility scores for fluorescence ratio images were significantly higher than for absorption images. The positive predictive value for classifying carcinomas by fluorescence ratio images reached more than 90%.

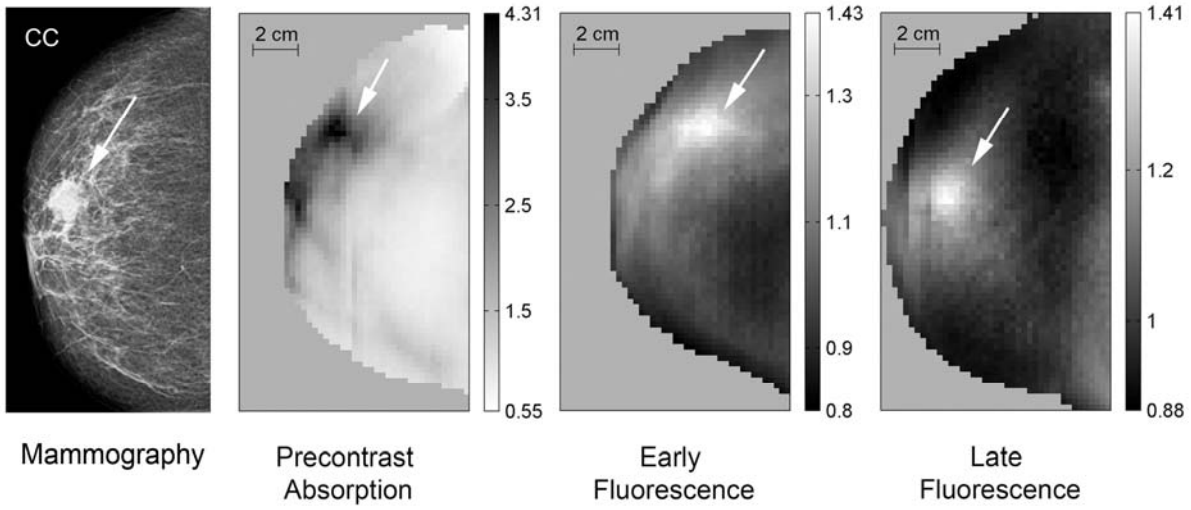

Fig. 1. Comparison of mammography, NIR precontrast absorption, NIR early and late fluorescence scans after application of ICG in a 68-years old woman with an invasive ductal carcinoma in her right breast. Tumor to background contrast enhances from the early to the late fluorescence scan (arrows). (Due to different positioning the location of the tumor is slightly shifted from the early to the late scan)

Conclusion

Capitalizing on early enhancement characteristics as well as on the extravasation of the dye through the wall of tumorous vessels in late fluorescence scans our data suggest that contrast-enhanced optical imaging is a promising technique to distinguish malignant from benign breast lesions.

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Invited

3D IMAGING TECHNIQUES AND THEIR PERSPECTIVES FOR CLINICAL USE

H. Schneckenburger[1,2]

[1]Hochschule Aalen, Institut für Angewandte Forschung, Beethovenstr. 1, 73430 Aalen, Germany
e-mail: herbert.schneckenburger@htw-aalen.de
[2]Institut für Lasertechnologien in der Medizin und Messtechnik an der Universität Ulm, Helmholtzstr. 12, 89081 Ulm, Germany

Abstract

3D imaging methods with laser scanning, structured or selective plane illumination are well established in fluorescence microscopy of cells and tissue samples. Although these techniques also have some potential in clinical practice, they have, so far, been limited to a few disciplines, e.g. dermatology [1] or ophthalmology [2]. On the other hand, 3D navigation systems [3, 4] as well as stereo vision [5] have been introduced into clinical practice. Therefore, a comparison of those systems with respect to applicability, resolution (and costs) appears to be cogent.

In addition, a combination of 3D imaging with spectral or temporal resolution is desired for many applications, e.g. tumour diagnosis based on intrinsic fluorescence or use of specific photosensitizers. While several in vitro applications on spectral imaging or fluorescence lifetime imaging microscopy (FLIM) are reported in the literature [6–8], clinical applications of these techniques are still rather limited. Nevertheless, they may have some larger potential, as depicted in the present paper.

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Invited

CLINICAL ASPECTS OF LASER TREATMENT OF BONE AND TEETH

R. Steiner

Institut für Lasertechnologien in der Medizin und Messtechnik an der Universität Ulm
Ulm, Germany, rudolf.steiner@ilm.uni-ulm.de

Pulsed infrared laser systems are well suited to ablate hard biological tissue like bone and tooth materials, enamel and dentin. But also urinary and kidney calculus can be disintegrated to facilitate natural elimination. Medical laser systems are in clinical use and the applications will be extended to other medical disciplines.

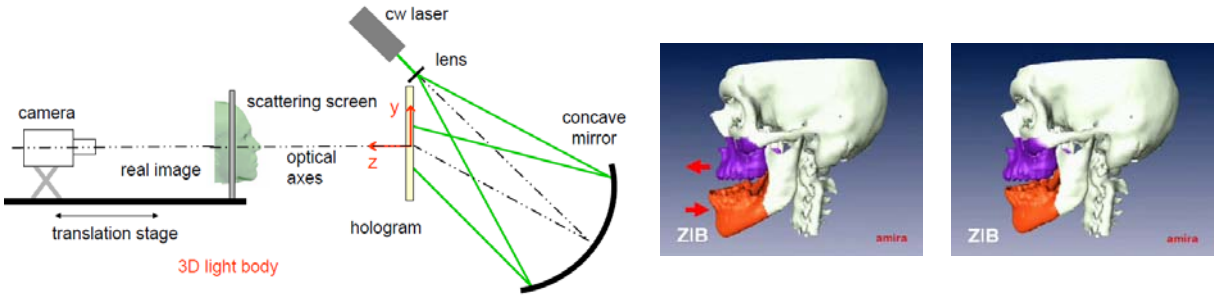
Modern laser systems are equipped with diagnostic tools and feedback control for save manipulation and removal of the target tissue. Also in combination with fast 3D imaging, like in oral and maxillofacial surgery, bone reconstruction can be optimised.

Some background information of the technologies used and examples of clinical applications will illustrate the useful clinical practice of such laser technologies.

Bone ablation

Due to high absorption of bone and dental material, the pulsed Er:YAG laser systems with a wavelength of 2.94 µm and short pulsed TEA CO_2 Lasers at 9,47 µm wavelength are suited for laser-osteotomy. Bone contains 26% water, 38% proteins, 2% lipids and 34% minerals. The main content of the minerals is Hydroxylapatite (80%) followed by Calcium carbonate (10%), Magnesium phosphate (1.5%) and Alkali salt (2,5%) [1]. The melting temperature is between 1350°C and 1540°C. Water content and Hydroxylapatite are the essential absorbers for the laser pulses. High absorption and short dwell time are responsible for efficient ablation and narrow zone of necrosis, necessary for a fast healing process.

Three dimensional cutting of bone with flexible formed cutting lines are preferable in maxillofacial surgery and the domain of laser application. At CAESAR in Bonn, Prof. Hering [4, 5] has developed a holographic method which allows the reconstruction of faces after bone destruction by cancer or also in forensic remodelling of faces [2]. The three-dimensional holographic data-set is calculated in 20 to 25 seconds. Such data together with an automated laser osteotomy is a powerful tool which has been developed and applied in clinical face reconstructions by Prof. Zeilhofer at Kantonsspital-Basel, Switzerland.

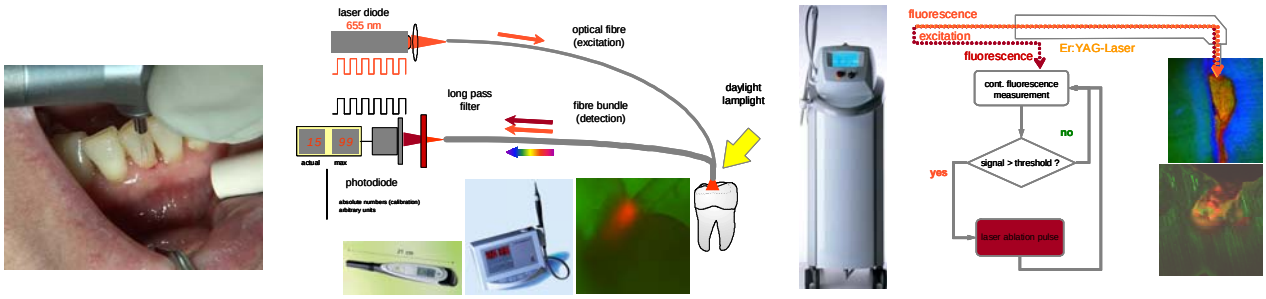


Scheme of the digitisation of the hologram (P. Hering, CAESAR)
and reconstruction (K. Zuse Institute, Berlin)

Lasers in dentistry

The disadvantage of the short pulsed CO_2 laser is the transmission of the beam by articulated arms. This makes the laser not flexible enough to be used in dentistry. However, the solid state Er:YAG laser with 2.94 µm wavelength can be transmitted through IR-fibres and is suitable for clinical routine. Meanwhile, several companies developed an Er:YAG laser for applications in dentistry, but the most flexible device has been developed by ILM and KaVo (Key-Pulse Technology). The laser with special changeable applicators can be used to remove caries, in endodontia and periodontics. Together with a diagnostic system [6] for caries diagnosis by red light excited fluorescence of caries bacteria, the therapy laser works autonomous which is necessary when the dentist does not see the site of action as in periodontics.

The diagnostic system of the DIAGNOdent has been integrated into the hand-piece of the therapy ER:YAG laser and the excited fluorescence intensity determines whether the laser is allowed to shoot or not.



a b c

a) Dental laser application in periodontics, b) caries diagnosis system.
c) scheme of autonomous Er:YAG laser system.

The continuous development of such dental applications guaranteed the clinical success and the diffusion of the laser systems into doctor's dental practice.

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Invited

PHOTODYNAMIC THERAPY OF THE MOST AGGRESSIVE FORMS OF MALIGNANT NEOPLASMS

E.Ph. Stranadko

State Research and Clinical Center for Laser Medicine
of the Ministry of Public Health and Social Maintenance of the Russian Federation
seph04@yandex.ru

Introduction

Currently, photodynamic therapy (PDT) is considered to be an acknowledged alternative technique for treating malignant neoplasms to surgical, radiation and chemotherapeutic techniques.

PDT is a locoregional organ preserving technique. In PDT 60% of the antitumoral effect is produced by vascular mechanisms, about 30% by the direct cytotoxic effect, and a little more than 10 % by immune mechanisms.

PDT opens wide possibilities for making various curative radical and palliative interventions when other medical approaches proved to be ineffective or are inapplicable at all. PDT efficacy in skin cancer has been proved by thousands of effectively treated patients. Our experience is based on more than 1 500 patients with malignant neoplasms of external and visceral localizations.

Goal

The goal of the research is study of PDT capabilities and efficiency for treating the most aggressive forms of malignant neoplsams: esophageal cancer, lung cancer with invasion into trachea and large bronchi, tongue and oral mucosa cancer, cancer of the large duodenal papilla and extrahepatic bile ducts, skin melanoma and its metastases (Table 1).

Table 1. Characteristics of patients with the most aggressive malignant tumors

Pathology	Number of patients	Age (average)	Men	Women
Esophageal cancer	40	43-85 (68)	33	7
Lung cancer	10	50-77 (62)	10	-
Tongue and oral mucosa cancer	76	26-88 (57)	50	26
Cancer of the large duodenal papilla and extrahepatic bile ducts	22	47-80 (60.5)	13	9
Primary melanoma and its metastases	18	40-86 (62.6)	7	11
TOTAL	166		113	53

In the last 19 years we have applied several home-made and foreign photosensitizes: Photohem (hematoporphirine derivative, Russia) in the dosage 1.5–5.0 mg/kg, Photosense (sulfonated aluminum phthalocyanine, Russia) 0.5–1.5 mg/kg, Photoditazin, Photochlorine, Radochlorine (chlorine e6 derivatives, Russia) 0.5–0.7 mg/kg, Photolon (chlorine e6 derivative, "Belmedpreparati", Belorussia) 1.3–2.0 mg/kg, Phoscan (Temoporfin, Biolitec Pharma Limited, Germany) 0.03–0.15 mg/kg.

Light sources were lasers (Russia) with wavelength 630 nm for Photohem, 670 nm for Photosense, and 662 nm for chlorine derivatives. Semiconductor lasers Cerelas PDT 652 (Germany) were also used. Light doses were 100–300 J/cm^2 for superficial irradiations, 100–300 J/cm of diffusor length in intralumenal irradiations of hollow organs when lightguides with cylindric diffusors were used, 200-300 J/cm^2 for intracutaneous melanoma metastases, 300 J/cm^2 for primary melanoma with Phoscan, and 900-9000 J/cm^2 for primary melanoma with other photosensitizers.

Results

Endoscopic PDT has been performes in 40 inoperable patients with esophageal cancer: 2 patients had cancer of stage I-II; however both of them were considered inoperable because of serious concomitant diseases. 2 patients had cancer recurrence in esophageal-intestinal and esophageal-gastric anastomoses with invasion into the lower-thoracic part of the esophagus. 36 patients had extended obstructive cancer of cervical, medial and lower thoracic parts of the esophagus of stage III-IY, including 4 patients after repeated recanalizations with Nd-YAG laser. 3 patients had cancer

recurrences in medial-thoracic part of the esophagus after chemoradiation therapy. 40 patients had 68 PDT sessions. 2 patients with early esophageal cancer had complete tumor resorption (CR) with recurrentless period more than one year and life-span more than 3.5 years. Disphagia in obstructive tumors of stage III-IV was reduced in all patients; during control X-ray examinations it was found that the esophageal lumen had enlarged up to 1-1.5 cm. In the case of disphagia recurrences after Nd-YAG recanalization of stenosing tumors PDT resulted in longer remission (6–7 months against 3–4 months) because PDT impairs the vascular mechanism and blood supply in residual tumors for longer period.

Endoscopic PDT was used in 10 inoperable patients having central lung cancer. 9 patients had primary lung cancer; 5 of them had bilateral lesions in both main bronchi and in the trachea. One patient had recurrent squamous-cell cancer in the stump of left main bronchi 10 months after extended pulmonectomy and postoperative gammatherapy. 10 patients took 20 PDT courses: 2 patients took one course, 6 two courses, and 2 patients three courses. Breathing disorder in obstructive tumors in the trachea and bronchi decreased in all the patients. The therapeutic effect lasted for 5–6 months.

PDT was performed in 76 patients with locally spread squamous-cell cancer (SCC) of the oral cavity who had 87 tumoral foci. 50 patients had PDT as monotherapy, 26 as a basic component in the complex treatment combined with distant gammatherapy (DGT) and neoadjuvant polychemiotherapy (ChT) with platina and 5- fluorouracil preparations. DGT and ChT added to PDT in locally-spread SCC of the oral cavity increased curative efficacy: CR rate went up from 56% in PDT as monotherapy to 84.6%, the rate of partial resorptions (PR) was 15.4%. All patients with lower lip cancer and oral mucosa cancer had CR. In the case of tongue cancer CR was seen in 14 out of 18 patients (77.8%), PR in 4 (22.2 %).

PDT was used for 22 patients with extrahepatic bile duct cancer (EHBDC), with complicated cancer of gallbladder (Mirrizi syndrome) and with cancer of large duodenal papilla (CLDP) (19 patients had adenocarcinoma of various differentiation degree; 3 patients had cancer without supplementary definitions). Photosensitizers (Photosense, chlorine derivatives) were injected intravenously to all patients. 22 patients underwent 31 PDT courses. Average life-span in patients after PDT was 2.5 years. Survival period, calculated with the actuarial test, was the following: 77.8% – one-year, 63.6% – three-year, and 38.2% – five-year. PDT in EHBDC cancer, in complicated forms of gallbladder cancer and CLDP cancer reduces cholestasis, improves the quality of life and prolongs life-span. Results of life-span after PDT treatment of the mentioned cancer locations are quite comparable with those after radical surgery and even exceed them compared to palliative surgeries. Retardation in tumoral growth after PDT and increased life-span in patients with residual tumors is explained by vascular PDT effect which causes vessel thrombosis in the residual tumors, thus impairing blood supply and promoting long stabilization of the process.

Melanoma is the most aggressive malignant tumor the rate of which is steadily going up while curative techniques are not always effective. PDT was done to 18 patients: 10 of them had primary melanoma, 3 recurrent melanomas, and 5 intracutaneous metastases. One patient had melanoma combined with metastases into the lungs, two patients had recurrences with local dissemination. 14 PDT causes were done with Photosense, 4 with Photohem, 12 with Photoditazin, and 4 with Photoscan. CR of the primary melanoma was seen in 10 patients who were followed up from 6 months to 12 years. One patient with multiple metastases of skin melanoma on the lower leg which was resistant to polychemiotherapy and repeated episodes of local dissemination had 15 PDT courses during 6 years. After PDT courses we could see resorption of melanoma metastatic foci in this patient up to 6 months without any adverse effects and complications. 4 patients had PR of their tumors. The data obtained confirmed high PDT efficacy for treating primary skin melanomas; PDT in intradermal melanoma metastases gives a good palliative effect with prolonged life-span in this severe pathology.

Conclusion

Even in the most aggressive malignant tumors resistant to traditional treatment PDT is often efficient and leads to patients' recovery. In incurable cancer forms which are resistant to traditional treatment, such as melanoma, extended obstructive esophageal cancer, lung cancer with invasion into the trachea and large bronchi, cancer of the large duodenal papilla and extrahepatic bile ducts, PDT helps to get a marked palliative effect, to improve the quality of life, and to prolong life-span in this group of patients.

OPTICAL IMAGING FOR MONITORING POST-TUR PATIENTS

O.S. Streltsova, E.V. Zagaynova, and **E.A. Tararova**

Nizhny Novgorod State Medical Academy, Russia
strelzova_uro@mail.ru

Introduction

Residual tumor risk in post-TUR patients with Ta,T1 bladder cancer is very high, amounting to 33-53% [1]. Cystoscopy is still the principal technique for diagnosing tumor recurrence. However, tissue edema, inflammation, bladder wall trabeculation, and post-operative alterations reduce diagnostic accuracy of studies. Repeated bladder TUR performed 2-8 weeks after the first operation helps to detect residual tumors in many patients (22–75%) at early stages [2]. However, the rate of detecting tumors that "have not been removed at first resection" in these 22–75% is analogous to that of early recurrences [2], which prejudices performing re-TUR. The use of fluorescence cystoscopy for re-TUR [3] does not solve the problem either, as in 55% of cases this results in removal of "non-tumorous" tissue [4]. Therefore, development and application of new diagnostic techniques for improving imaging of recurrent tumor lesions in the bladder and safe monitoring of the area of surgery, scar included, are very important tasks.

The goal of the research is justification of a possibility to use optical imaging for analysis of the area of postoperative scar aiming at re-TUR minimization.

Materials and methods

The cross polarization OCT (CP OCT) that allows verifying processes in subepithelial connective-tissue bladder wall structures was first used for monitoring mucosa of post-TUR bladder from 2 to 144 weeks in 66 patients. 295 cystoscopies were made, 165 (55.9%) of which required additional studies with CP OCT for detecting visually altered zones of mucosa suspicious for neoplasia. 1169 images were acquired total. Scars up to 8 weeks old were regarded to be early, more than 8 weeks – old.

Results

Residual tumors were detected in 4 cases in patients with pT2 tumors. Postoperative scars were not found in 31 cases, were detected in 79 cases, and in 41 cases they had conventional form, which was confirmed by CP OCT studies. Areas visually suspicious for neoplasia in the perifocal zone near the scar were revealed in 24.8% of cases (n-41), on the scar in 23% (n-38), and outside the zone of surgery in 54 cases (32.7%).

Visually suspicious areas that require additional CP OCT study, number of cases (%)		
165 (55.9%)		
Outside the zone of surgery	Perifocal zone	Suspicious scar
54(32.7%)	41(24.8%)	38(23%)
Surgery indicated by CP OCT – 36 (1.8%)		
18(33.3%)	8 (19.5%)	10 (26.3%)

According to CP OCT data, 36 (21.8%) of 165 visually suspicious zones had suspicious CP OCT images and were resected, i.e., **78.2%** of zones visually suspicious for neoplasms did not require surgery. Tumor growth was detected histologically only in 28 of 36 cases, which was 77.7%, in 8 cases dysplasia was detected.

Analysis of early scars with erosive surface (n-5) (fig. 1a) and study of the ulcer edge (n-3) (fig.1 b) with CP OCT allowed tumor to be excluded and monitoring to be continued (fig. 1c). In 10 (26.3%) cases CP OCT images in co-polarization were structureless or had disordered structure and in cross-polarization weaker or focally weaker signal was visualized, which corresponded to neoplasms and demanded scar resection (fig. 1d).

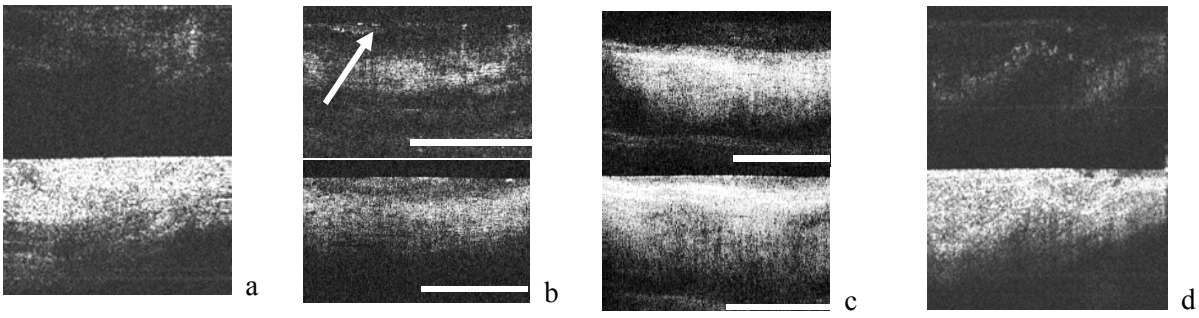

Fig. 1. CP OCT images: a) ulcer center, moderate intensity signal from connective-tissue structures in cross-polarization (7 weeks after surgery); b) ulcer edge, layered structured tissue; salts in the epithelium above forming scar (shown by arrow); c) the same scar 1 year later; d) focal epithelial dysplasia in the scar area, acanthosis. In cross-polarization, signal from subepithelial structures is weaker, tissues are disordered in the longitudinal direction, histological samples after high-malignancy urothelial cancer re-TUR show no signs of invasive growth

Conclusion

The CP OCT imaging technique allows verifying tumor and scar tissue, thus minimizing re-TUR of urinary bladder.

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Invited

TOWARDS RADIATION THERAPY WITH LASER-DRIVEN ION BEAMS

J.J. Wilkens

Department of Radiation Oncology, Technische Universität München, Klinikum rechts der Isar
München, Germany, wilkens@tum.de

Radiotherapy with proton and ion beams

Besides surgery, radiation therapy is a key method for treating tumour patients with localized disease. Over the last years, advances in research and technology led to significant improvements in various fields of radiotherapy, resulting in higher cure rates and less side effects in normal tissue. The majority of radiation treatments for tumours in humans is currently given by ultra-hard x-ray beams generated by clinical linear electron accelerators. Within the last decade, the clinical interest in high-energy protons or heavier ions as a promising alternative has risen clearly (see for example [1–3]). Compared to the standard x-ray treatment, this so-called particle or ion beam therapy can deliver better dose distributions with less dose burden in normal, healthy tissue. The therapeutical use of ion beams was proposed by Wilson in 1946 [4], and the first patients were irradiated in the 1950s and 1960s in the USA (Berkeley and Harvard), Sweden (Uppsala) and the former Soviet Union (Dubna and Moscow). Since then, more than 70 000 tumour patients have been treated with ions all over the world (85% with protons, 15% with heavier ions, mainly carbon). The number of proton/ion beam facilities is increasing, and especially in the last few years several new hospital based ion accelerators started their operation (e.g. the Heidelberg Ion Beam Therapy Center in Heidelberg/Germany), while some more are under construction. Rather than the high energy physics laboratories, where the first patients were treated, these new machines are dedicated only to medical applications and can provide a patient friendly environment, higher patient throughput and research facilities in the fields of oncology and medical physics. Turn-key solutions are available from several commercial vendors.

Clinically, the primary indications for proton therapy are uveal melanoma, various paediatric tumours as well as chordomas and chondrosarcomas of the skull base. Due to the lower integral dose in normal tissue, one could argue that proton therapy is advantageous compared to photon irradiation in almost all situations, although there are only very few clinical studies to confirm this. In any case, proton therapy has successfully been applied to many other tumour entities like prostate cancer, non-small-cell lung cancer, head-and-neck tumours and meningeomas. Heavier ions like carbon ions were mainly used to treat chordomas and chondrosarcomas of the skull base and malignant salivary gland tumours. Again, a variety of other indications like prostate, head-and-neck, lung and liver are currently being investigated with respect to their suitability for carbon ion therapy [1].

New accelerator concepts

A major drawback of ion beam therapy is that the required technology for ion acceleration (large scale cyclotrons or synchrotrons) and beam lines is complex and very costly. As a consequence, this type of therapy is still limited to roughly 30 centres worldwide. Currently there are several approaches how to make this technology more compact and cost-efficient. Some companies are working on commercial solutions for compact, dedicated treatment machines that are still based on conventional accelerator technology, e.g. a superconducting cyclotron for carbon ions (IBA/Belgium, www.iba-worldwide.com), a relatively compact synchrotron for proton beams (ProTom International Inc./USA, www.protominternational.com) or a proton synchro-cyclotron mounted on a gantry as a single-room solution (Still River Systems/USA, www.stillriversystems.com). Alternative acceleration concepts are the so-called dielectric wall accelerator [5] and particle sources based on laser-plasma acceleration using intense, ultra-short laser pulses [6]. Compact laser-based ion sources could dramatically increase the availability of high-energy proton and carbon ion beams, and provide particle therapy to a broader range of patients.

Laser-driven ion beam radiation therapy

One huge advantage of laser based ion therapy units compared to conventional technology could be a compact gantry that does not need large and heavy bending magnets to deflect the ion beam. Instead, the laser beam would be guided by mirrors through a compact gantry structure to hit the target in the

treatment head close to the patient. Ideally, this structure should not be much larger than electron linacs currently used in radiotherapy. At present, the achievable energies for laser-accelerated particles are not high enough for therapeutic applications, and the detailed properties (fluence rates, energy spectra, divergence etc.) of future beams are not precisely known yet. Therefore there are not even prototypes of such irradiation units available. Various groups (e.g. at the OncoRay centre in Dresden-Rossendorf/Germany, at the Munich-Centre for Advanced Photonics in Munich/Germany, and at the Photo-Medical Research Center in Japan) are currently investigating the required physical, technological and biological basis for laser-based radiation therapy with proton or carbon ion beams.

The Munich-Centre for Advanced Photonics

The interdisciplinary research cluster "Munich-Centre for Advanced Photonics" (MAP) focuses on laser-driven particle beams both from a fundamental physics point of view as well as with respect to applied medical physics and radiobiology with the long term goal of developing a laser-based particle therapy unit. In particular, the ion acceleration process itself is studied using ultra-thin diamond-like carbon foils as targets, and a laser-driven biomedical beamline for radiation biology and dosimetry is under construction. One important task is to study potential variations of the relative biological effectiveness (RBE) due to extremely high dose rates within each particle bunch generated by a single laser shot. The energy and number of particles per shot and the dynamic range and precision to control it on a shot-by-shot basis are essential for a safe delivery and for treatment planning, where strategies to find robust and efficient treatment plans are being investigated to keep the required number of laser shots to a minimum [7]. Additionally, appropriate methods for beam delivery including lateral and axial beam shaping for highly pulsed beams have to be developed in order to design clinical treatment units for laser-driven particle beams. Axial (in depth) beam delivery could be complemented with “spectral shaping” techniques [8] in an energy selection system to deliver a full spread-out Bragg peak to a macroscopic volume within nanoseconds.

Towards clinical applications

A major milestone towards the therapeutic application of these new beams has been demonstrated by the first cell irradiations using laser-accelerated protons [9, 10]. With the progress on the physics side (e.g. the realization of new acceleration regimes), we expect higher energies being available soon. Biological investigations will then shift to pre-clinical studies in mice and finally towards clinical treatment units for cancer care.

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MATCHING OF DIFFUSE REFLECTANCE SPECTROSCOPY DATA AND CLINICAL OUTCOMES IN BREAST CANCER

S. Zinovjev[1], **A. Orlova**[3], **B. Aboghessimengane-Zo'o**[2], **G. Golubiatnikov**[3], **A. Maslennikova**[1], **M. Kirillin**[3], **N. Shakhova**[3], **S. Belkov**[4], **T. Lubynskaja**[4]

[1]Nizhny Novgorod State Medical Academy, Russia, swet.ssk@yandex.ru
[2]N.I. Lobachevsky State University of Nizhny Novgorod, Russia
[3]Institute of Applied Physics RAS, Nizhny Novgorod, Russia
[4]BIOFIL Ltd., Sarov, Russia

Breast cancer is the most common form of malignant tumor in women with more than 200000 cases diagnosed worldwide each year [1]. The standard procedures to confirm the diagnosis are X-ray mammography and core biopsy in which a needle is injected into the breast and tissue sample is removed. These methods may provide information concerning location and structure of the tumor, but are unable to estimate its biological properties. The limitations of the current breast cancer diagnostic procedures gave an impetus to develop minimally invasive immediate diagnostic systems, giving information about tumor biological properties, in particular, its oxygen status. The optical scattering and absorption properties of tissue have been demonstrated in clinical trials to be sensitive to the tissue type and state. Diffuse reflectance spectroscopy (DRS) was shown to be sensitive to the size and structure of the subcellular components that change upon transformation to premalignant or malignant condition [2, 3]. Also it was demonstrated that DRS is a quantitative tool to measure oxygenation levels in the vascular compartment of breast cancers in vivo [2]. In turn, oxygen status is considered to be one of prognostic factors. The objective of this study was to compare disease outcome and data obtained by diffuse reflectance spectroscopy procedure in patients with breast cancer.

Materials and methods

From 2004 to 2005 one hundred forty six female patients (88 malignant tumors, 58 benign lesions) were examined with DRS using BioTelligent Inc. probe [3]. To collect diagnostic data the probe was inserted into the breast and navigated to the suspicious lesion. Over 1000 data sets are collected per one insertion. Detected spectra variations indicate changes in the tissue state so that data from normal and abnormal tissue are obtained in one patient. The two algorithms for computerized discrimination of the tumor type have been developed based on the idea of correlation of the experimental spectrum with the test curve corresponding to a specific tumor. This procedure results in splitting the total set of experimental signals for each tumor type into a few subtypes M1, M2 and M3. Disease outcomes in 28 patients (median age 46 years) with different optical subtypes of breast cancer were analyzed. Median follow-up was 62 months.

Results

Disease outcomes were analyzed depending on clinical characteristics and spectra type. 16 patients had $T_{1\text{-}2}$ tumors, 12 patients had T_3 tumor. Overall survival, disease-free survival and progression type were considered. Sixteen patients had M1 spectrum type, two of them developed progression. One patient had local recurrence a year after the treatment, the second developed distant metastases in three years. In the case of M2 spectrum, 1 patient, and M3 spectrum, 11 patients are alive without progression for 5 years.

Conclusion

We failed to determine the correspondence between spectrum type and disease outcome. More detailed analysis of component distribution of the tumor tissue is necessary to obtain information concerning the biological properties of the neoplasm and their influence on disease prognosis.

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