Spectrochimica Acta Part B 146 (2018) 106–114

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Using LIBS to diagnose melanoma in biomedical fluids deposited on solid substrates: Limits of direct spectral analysis and capability of machine learning

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ARTICLE INFO

Keywords:
LIBS
Diagnosis of cancer
Melanoma
Machine learning
Biological fluid analysis
Effect of substrate

ABSTRACT

Diagnosis is crucial to increase the success rate of cancer treatments as well as the survival rate and life quality of patients, in particular for forms of cancer that remain largely asymptomatic until metastasis. Methodologies that allow the diagnosis of early-stage tumors as well as the detection of residual disease have the potential to improve cancer control and help monitor therapeutic outcomes. In this work, we report a Laser-Induced Breakdown Spectroscopy (LIBS) approach to early diagnosis of a form of skin cancer, melanoma, based on the analysis of biological fluids (blood and tissue homogenates) harvested from diseased mice and healthy controls. We acquired femtosecond LIBS spectra and used two different approaches for the analysis: through comparison of the emission intensity of selected analytes in healthy and diseased samples; and by using machine learning classification algorithms (LDA, Linear Discriminant Analysis; FDA, Fisher Discriminant Analysis; SVM, Support Vector Machines; and Gradient Boosting). We also addressed the effect of substrates on the analysis of liquid samples, by using four different substrates (PVDF, Cu, Al, Si) and comparing their performance. We show that with a combination of the most appropriate substrate and algorithm, we are able to discriminate between healthy and diseased mice with accuracy up to 96% while direct analysis of LIBS spectra did not provide any conclusive results. These series of results demonstrate that carefully designed LIBS measurements combined with machine learning can be a powerful and practical approach for the diagnosis of cancer.

1. Introduction

Cancer indicates a class of diseases related to abnormal cell growth in one organ or tissue, with the potential to spread to other parts of the body. Many research and medical efforts are ongoing to efficiently diagnose and fight cancer, but the various forms of this disease are still one of the leading causes of death worldwide. Fighting cancer is very complex, in that it necessarily involves many different aspects, such as: investigating the causes of its onset; developing minimally invasive, targeted, and, in the future, personalized therapy approaches; promoting prevention practices; implementing screening tests for early diagnosis. The latter is a key task, as it is well-documented that detecting the onset of the disease during its early stage of development can significantly improve significantly the success of treatments and ultimately the survival rate and quality of life of patients. This issue is particularly critical for kinds of cancer that develop in the absence of specific symptoms and can go largely unnoticed until they metastasize, such as epithelial ovarian cancer (EOC), pancreatic cancer, and melanoma [1,2].

Developing large-scale screening tests is one of the most efficient strategies for early diagnosis of this kind of tumors. Ideally, such tests should be rapid and minimally invasive, user-friendly, accurate (low number of false positives and false negatives), and easy to integrate in point-of-care structures, so as to reach and monitor large numbers of people on a periodic basis. Laser-Induced Breakdown Spectroscopy (LIBS) is characterized by well-known practical advantages, which

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https://doi.org/10.1016/j.sab.2018.05.010
Received 8 March 2018; Received in revised form 23 April 2018; Accepted 11 May 2018

include limited sample preparation, fast multi-elemental response, compact instrumentation, possibility of in situ analyses, and versatility, all of which can contribute to making this technique a powerful tool in the fight against cancer.

Despite being essentially an atomic spectroscopy technique, and as such not an obvious choice for the diagnosis of diseases that proceed through an abnormal proliferation of cells, LIBS has proved useful to distinguish between biopsied cancerous tissues and adjacent healthy ones, thanks to differences in the content of trace elements. In particular, previous studies have almost consistently shown that cancerous lesions have a different alkaline and alkaline earth metals content than healthy tissues [3–6].

In [7], Melikechi et al. proposed for the first time to develop a LIBS-based "liquid biopsy" approach for the early detection of cancer, i.e. the analyzed samples were not tissues (either biopsied or harvested from laboratory animals), but sera. In [7], we acquired femtosecond-LIBS spectra of mice sera taken from animals with EOC and healthy controls of three different age groups and deposited on a solid substrate. The LIBS spectra were then fed to two different classification algorithms that were shown to be useful for the discrimination of sera from mice with cancer and healthy ones with a maximum accuracy around 80%. Recently, Chen et al. have adopted essentially the same experimental and computational approach to the diagnosis of lymphoma and multiple myeloma in human serum, and have obtained classification accuracies close to 100% [8].

In the present work, we obtain LIBS spectra of blood serum and tissue homogenates harvested from a pre-clinical model of melanoma (B16-F10), an often asymptomatic and highly aggressive type of skin cancer (see for example [9,10]). We aim for three goals. First, to investigate if analogous conclusions to those obtained on melanoma [6] can be drawn from this work. In other words, is the enrichment in Mg and Ca in melanoma lesions with respect to healthy skin reported in skin tissues also present in biological fluids? Second, we compare the performance of four different classification algorithms (LDA, Linear Discriminant Analysis; FDA, Fisher Discriminant Analysis; SVM, Support Vector Machines; and Gradient Boosting). Third, for the first time we address the effect of four different solid substrates (Polyvinylidene fluoride (PVDF); Cu; Al; Si) on the signal-to-noise ratio of LIBS spectra of biological fluids and on the classification accuracy. We show that the classification accuracy varies significantly with the type of algorithm selected and of substrate used. In this particular study, the best results are obtained with Gradient Boosting using a copper substrate, which yields a classification accuracy close to 100%. In essence, this work demonstrates the power of this approach even when a direct LIBS spectral analysis of cancerous and non-cancerous samples does not provide any indication of distinguishability.

2. Experimental setup

The laser source and the spectroscopic system have been previously described [7], and will only be briefly recalled here. The plasma was produced by focusing a 150-fs Ti-Sapphire laser (Clark-MXR, Model 2210, wavelength = 775 nm) on the samples, through a fused silica biconvex lens (focal length = 50 mm, focused spot size = 100 μm). The samples were mounted on a motorized and computer-controlled x-y translation stage (scanning speed = 0.35 mm/s), to ensure that each laser shot would ablate a fresh surface. Measurements were performed in an experimental chamber filled with slight over-pressured He (762 Torr), in order to reduce the spectral interference from air elements and obtain a more persistent and bright plasma. The optical emission from the plasma was collected by a fiber collimation lens 45° with respect to the laser beam and focused onto a 50 μm core-diameter optical fiber, and coupled with the spectroscopic acquisition system. The latter comprised an Echelle spectrograph (Andor Technology, ME 5000) for wavelength dispersion and a thermoelectrically cooled iStar Intensified Charge Coupled Device (ICCD) camera for radiation detection (Andor Technology, DH734-18F O3). We acquired spectra with 50 ns starting delay time after the laser pulse and 700 μs integration time.

We analyzed two different kinds of biological fluids, obtained from mice with melanoma and healthy controls: blood serum and homogenates of three different tissues (lungs, spleen, lymph nodes), that were prepared following the protocols described later on.

In the first series of experiments, we deposited 5 μl drops of each of the biological fluids on PVDF membranes, and dried them for 10 min prior to the LIBS analysis, using a tungsten IR lamp. The laser energy we used for this series of experiments was 1.44 mJ. In the second series of experiments, we selected only one of the biological fluids (blood serum) and studied the effect of different substrates on the LIBS spectra and classification accuracy. The sample preparation procedure was the same, and the three employed substrates were Cu, Al, and Si. Prior to depositing serum, the two metallic substrates were mechanically polished, and all three substrates were rinsed and sonicated in 2-propanol. The laser energy we used for this series of experiments was 1.20 mJ. We constantly monitored the laser energy during the spectra acquisition, and spectra with intensity lower than a given threshold were automatically rejected, so as to improve the signal-to-noise ratio.

3. Sample preparation

3.1. Mice

Mouse experiments were performed in accordance with institutional guidelines under a protocol approved by the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Animal Care and Use Committee. All mice were maintained in a pathogen-free facility according to the National Institutes of Health Animal Care guidelines. C57BL/6J mice (females, 6 to 10 weeks old) were purchased from The Jackson Laboratory.

3.2. Cell line and tumor implantation

The B16-F10 mouse melanoma line was originally obtained from I. Fidler (MD Anderson Cancer Center, Houston, TX). Cells were maintained in RPMI 1640 containing 7.5% fetal bovine serum (FBS) and L-glutamine. For the tumor implantation, 2×10^5 viable B16-F10 cells in 100 μl of phosphate-buffered saline (PBS) were injected intradermally into the right flank of C57BL/6 mice.

3.3. Sample preparation protocol

Mice were euthanized 2 weeks after tumor implantation, and four different tissues (lymph nodes; spleen; lungs; blood serum) were harvested from tumor-bearing or non-tumor-implanted mice. Blood serum was analyzed as such, while tissues were mechanically dissociated using a PowerGen 125 tissue homogenizer (Fisher Scientific) in a protein lysis buffer (LB), with the following composition: 0.01 M Tris-HCl, 0.15 M NaCl, 0.01 M $MgCl_2$, 0.5% NP-40 in distilled water.

4. Results and discussion

Previous LIBS studies for cancer diagnosis have almost consistently shown that cancerous tissues have a different elemental composition than healthy tissues, these differences being mostly due to the alkaline and alkaline earth metals content. Ca and Mg levels have usually been found to be higher in the cancer-affected areas, with at least four different kinds of tumor (colorectal cancer [3], breast cancer [3,4], canine hemangiosarcoma [5], and melanoma [6]). An exception to this trend was observed in [11], where no Ca and Mg enrichment was found in Malignant Pleural Mesothelioma (MPM), but instead MPM tissues resulted to be enriched in P and O and depleted in Zn and Cu. Following these observations, we set out to determine whether statistically