Coordination Chemistry Reviews 358 (2018) 70–79

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

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

Review

Elemental imaging using laser-induced breakdown spectroscopy: A new and promising approach for biological and medical applications

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ARTICLE INFO

Article history:
Received 15 September 2017
Received in revised form 13 November 2017
Accepted 9 December 2017

Keywords:
Elemental imaging
Laser-induced breakdown spectroscopy (LIBS)
Biological tissues
Medical diagnostics
Metal nanoparticles

ABSTRACT

Biological tissues contain various metal and metalloid ions that play different roles in the structure and function of proteins and are therefore indispensable to several vital biochemical processes. In this review, we discuss the broad capability of laser-induced breakdown spectroscopy (LIBS) for *in situ* elemental profiling and mapping of metals in biological materials such as plant, animal and human specimens. These biological samples contain or accumulate metal species and metal-containing compounds that can be detected, quantified, and imaged. LIBS enables performing microanalysis, mapping and depth profiling of endogenous and exogenous elements contained in the tissues with a parts-per-million scale sensitivity and microscopic resolution. In addition, this technology generally requires minimal sample preparation. Moreover, its tabletop instrumentation is compatible with optical microscopy and most elements from the periodic table. Specifically, low- and high-atomic-number elements can be detected simultaneously. Recent advances in space-resolved LIBS are reviewed with various examples from vegetable, animal and human specimens. Overall, the performance offered by this new technology along with its ease of operation suggest innumerable applications in biology, such as for the preclinical evaluation of metal-based nanoparticles and in medicine, where it could broaden the horizons of medical diagnostics for all pathologies involving metals.

Contents

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https://doi.org/10.1016/j.ccr.2017.12.006
0010-8545/

1. Introduction

Over the last two decades, laser-induced breakdown spectroscopy (LIBS) has become a recognized and valuable analytical spectroscopic technique for analyzing the elemental nature of any type of sample, such as a solid, liquid or gas [1,2]. Many additional advantages of LIBS are acknowledged, such as its simplicity and ability to work at room temperature and under ambient pressure conditions; its ability to detect almost every element on the periodic table, including low- and high-Z elements [3]; its high sensitivity reaching the parts-per-million scale for most of the elements; its high dynamic range of detection (from major to trace amounts); and its standoff capabilities [4]. The accessible resolution is ultimately limited by the diffraction limit, which enables reaching the micrometer scale. In addition, since vacuum conditions are not required, there are no sample size or shape restrictions.

LIBS reveals the elemental composition of materials from a single-shot microanalysis (reviewed in [5]) to 2-D characterization (spatial mapping). This technology also has a 3-D capability (including depth profiling) [3]. These common characteristics, together with its table-top instrumentation and fast operation, render LIBS imaging a promising technology for elemental investigations in the fields of biology and medicine. At first, LIBS analysis was mainly developed to analyze the elemental composition of hard materials, with clear industrial and geological applications [4,6–8]. The capability of space-resolved LIBS for studying biological materials was first demonstrated on mineralized or calcified specimens such as gallstones [9], bones and teeth [10,11]. The possibility of imaging soft tissues via LIBS was demonstrated very recently. This development was limited by both the softness and the highly heterogeneous nature of biological tissues as well as the difficulty of mastering laser ablation on such materials.

The capability of LIBS (not space resolved) for biomedical applications was first demonstrated after a matrix transformation (*i.e.*, tissue mixing, drying, and pelleting), which was employed to improve the laser-ablation efficiency and therefore the LIBS signal-to-noise ratio [2]. Different LIBS instruments equipped with nanosecond or femtosecond lasers may be employed, for single or double pulse analysis [12]. In the related literature, LIBS was used to discriminate between normal and malignant canine hemangiosarcoma tumor cells [13] and to diagnose breast cancer [14], colorectal cancer [15], and melanoma [16]. In the last study, the authors measured the elements both in homogenized pellets and tissues obtained from excised skin samples of melanoma-implanted animals. Their results indicated that Mg and Ca were appropriate biomarkers for discriminating melanoma from normal skin, suggesting a potential direct clinical application of LIBS for human tumor diagnosis [16]. Recently, an innovative approach based on an elemental encoded particle assay coupled with femtosecond-LIBS was developed to improve cancer blood biomarker detectability [17]. In plant biology, LIBS was successfully used to discriminate healthy tobacco leaves from Tobacco Mosaic Virus-infected ones [18]. Finally, LIBS was tested to identify different tissues in real time during laser-based surgery. Using *ex vivo* porcine tissues, the authors successfully identified and classified different target tissues (*i.e.*, fat, nerve, muscle and skin) with high sensitivity and specificity [19,20]. Similarly, in hepatic copper (Cu)-accumulation-related disease, *i.e.*, Wilson's disease, LIBS correctly identified diseased tissues based on the Cu/C content ratio, which discriminated pathological liver biopsies from normal tissue [21].

The aim of this review is to present recent advances in space-resolved LIBS, especially imaging, for biological soft materials. After a short description of the importance of metals in biomedicine, a brief review of standard imaging technologies will be presented before focusing on the capabilities of the LIBS technology for plant, animal and finally human specimens.

2. Metals in bio-medicine and conventional imaging strategies

In biology, metals are essential actors in most of the functions of DNA, RNA and proteins and even help control epigenetic modifications [22]. In other words, metals govern most indispensable life processes. The redox-inactive alkali and alkali earth metals, notably sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca), are dynamic entities that convey signals through fast and orchestrated movements and exchange of metal ion pools. Redox-active transition metals such as zinc (Zn), copper (Cu) and iron (Fe) are known cofactors involved in the maintenance of structural or catalytic roles. Some of them, when dysregulated, can trigger oxidative stress and damage [22,23]. Consequently, metals have multiple biological functions, ranging from catalytic, regulatory, structural, or signaling roles.

Metal homeostasis must be maintained by coordinated uptake, trafficking and efflux pathways that place the required amount of the correct metal at the appropriate place and time in the cell [24]. Analyzing chemical elements, especially metals, *in situ* in normal or diseased tissues is of considerable interest to determine whether they exist at their expected normal concentrations. In general, metal deficiency and excess strongly affect tissues and may lead to severe, if not deadly, diseases.

Some metals are known to be toxic and harmful to living organisms, whether plant or animal. Twenty-six elements from the periodic table were recently reported to be essential for most forms of life (Fig. 1). However, more than 20 additional non-essential chemical elements are naturally detected in humans. Importantly, the nature and concentrations of non-essential elements are highly variable among individuals, mainly depending on the tissue of interest and lifetime personal exposure [25].

Due to the fundamental importance of metals in biology, their *in situ* visualization plays a major part in describing and understanding their biological role in depth. The spatial distribution of specific metals and metal-based compounds is as important as their chemical properties, because both their concentration and localization change in biological systems, and their transport and compartmentalization is critical for effective utilization. The determination of elemental distributions (imaging or mapping) in biological sample surfaces has been of interest for a long time, and new technologies are now enabling such detection in the biological sciences [26,27].

Most of the current imaging techniques rely on (i) methods that employ metal-selective probes/chemical sensors; (ii) mass spectrometric detection; or (iii) "beam" methods that employ light/lasers, electrons, X-rays or energetic particles to measure characteristic radiation [28].

Several metal-selective probes or chemical sensors can be used for imaging metal ions such as Cu^{2+} [29,30], Fe^{3+} [30], Zn^{2+} [31], Cs^{+} [32], Al^{3+} [33] or heavy metal ions such as Ag^{+} or Hg^{2+} [34]. Mass spectrometric imaging of metals flourished following the integration of solid sampling techniques with element-specific detection techniques such as inductively coupled plasma-mass spectrometry (ICP-MS). Although laser ablation (LA)-ICP-MS has currently reached a technological barrier preventing practical sub-micron imaging, other MS-based methods, such as nano-secondary ion mass spectrometry (SIMS), are gradually providing the means to image metals at the subcellular level [35].

Multiple abnormalities occur in the homeostasis of essential endogenous brain bio-metals (namely, the late first-row transition metals Fe, Cu and Zn) in age-related neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, Huntington's