2020
NANOCON®

CONFERENCE PROCEEDINGS

12th International Conference
on Nanomaterials - Research & Application

October 21 - 23, 2020
Brno, Czech Republic, EU

NANOCON

TANGER Ltd.
The Czech Society for New Materials and Technologies
Regional Centre of Advanced Technologies and Materials

12th International Conference on Nanomaterials - Research & Application

CONFERENCE PROCEEDINGS

October 21 - 23, 2020
Brno, Czech Republic, EU

ISBN 978-80-87294-98-7

ISSN 2694-930X

NANOCON 2020 **Conference Proceedings**

Virtual Conference

Different Authors

October 21 - 23, 2020 Brno, Czech Republic, EU

Issued by: TANGER Ltd., Keltickova 62, 710 00 Ostrava, Czech Republic, EU

Edition: 1st Edition, 2020

Number of pages: 569

METTALOTHIONEIN SENSING USING MOLECULARLY IMPRINTED POLYMERS COUPLED LASER ABLATION INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

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https://doi.org/10.37904/nanocon.2020.3749

Abstract

In this work, we report a facile method for a detection of mettalothionein (MT) - potential biomarker of tumor diseases. Combination of laser ablation inductively coupled plasma coupled to mass spectrometric detection (LA-ICP-MS) with sample pretreatment by magnetic particles modified by molecularly imprinted polymers (Mag-MIP) enables selective and sensitive protein detection and quantification. After Mag-MIP MT extraction, elements specific for the protein (S, Zn and Cd) were detected in healthy pig skin and pig melanoma tissue using MeLiM (Melanoma-bearing-Libechov-Minipig) model. It was found that levels of detected elements were significantly elevated in melanoma tissue compared to healthy skin. Currently, the investigation of MT dimerization in cancer progression is carried out taking advantage from easily controllable *in vitro* MT dimerization which is used for simple preparation of selective MIP extraction phase. Unlike other recognition elements, which are not always available for target analyte (e.g. antibodies), MIPs can be easily prepared as required. Moreover, magnetic particles as a MIP substrate enable elegant extraction approach and LA-ICP-MS provides extremely sensitive detection. Therefore, Mag-MIP-LA-ICP-MS is a unique cross-border combination of molecular and elemental analysis.

Keywords: Metallothionein, Molecularly imprinted polymers, Magnetic particles, LA-ICP-MS

1. INTRODUCTION

Metallothioneins (MTs) are a class of low molecular-weight (6 to 7 kDa) and cysteine-rich proteins. MTs are intracellular and ubiquitous in eukaryotes, and have specific structural characteristics giving them potent metal-binding and redox capabilities [1], due their high affinity to metals, MTs participate in the regulation of cellular metabolism of metals like zinc and copper, in detoxification of toxic metals like copper, cadmium and mercury, and in protection of cells against reactive oxygen species and alkylating agents [2]. MTs participate in the carcinogenic process, which include the generation of reactive oxygen species, oxidative DNA damage, genomic instability and others [3].

Malignant melanoma is one of the most dangerous and deadly forms of cancer, being responsible for 1.2 % of all cancer deaths in the European Union. Malignant melanomas often go unnoticed, it is characterized by early metastasis and poor prognosis, and its incidence is increasing each year [4,5]. For immediate therapy and increase of survivors of melanoma, evaluation of histopathological and clinical parameters is important and mainly, the identification of potential biomarkers plays a critical role [6].

Molecularly imprinted polymers (MIPs) are synthetic materials with artificially generated recognition sites able to selectively interact with target molecules. Currently, MIPs are attracting widespread attention due to their properties such as flexibility, variability, high chemical and mechanical stability, relatively low cost and

simplicity in preparation [7,8]. The use of nanomaterials - magnetic nanoparticles - in combination with MIPs provides large surface-to-volume ratio and well defined shape; moreover possibility of rapid separation of magnetic particles by the aid of an external magnet is favorable [9].

In this work, a method combining laser ablation inductively coupled plasma mass spectrometric detection (LA-ICP-MS) with sample pretreatment by magnetic particles modified by molecularly imprinted polymers (Mag-MIP) was developed. The suggested approach enables selective and sensitive protein detection and quantification, especially detection of MT. After Mag-MIP MT extraction, elements specific for the protein (S, Zn and Cd) were detected as this information is essential for further development of effective and timely diagnostic tools for melanoma.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Dopamine hydrochloride, Trizma® base and acetic acid were purchased from Sigma-Aldrich (MO, USA) in ACS purity. MT from rabbit liver was obtained from Enzo Life Science (NY, USA). Dynabeads™ MyOne™ Silane magnetic particles and sodium tetraborate decahydrate were purchased from Thermo Fisher Scientific (MA, USA).

2.2. Preparation of magnetic MIPs

Briefly, 50 µl of magnetic nanoparticles (MPs) (40 mg·mL^{-1}) were washed by 200 µL of 20 mM TRIS (pH 8.5) for three times. MT (1 mg·mL^{-1}) was dissolved in 20 mM TRIS (pH 8.5) and 400 µL of solution was added to the washed MPs for preparation of Mag-MIPs. To prepare non-imprinted polymers (NIPs) used as a control, only 400 µL of 20 mM TRIS (pH 8.5) was added to the washed MPs. The mixtures were stirred for 1 hour and then 100 µL of dopamine (17.5 mg·mL^{-1}) dissolved in 20 mM TRIS (pH 8.5) was added and the reaction was continued overnight at room temperature. The product was collected using external magnetic field, supernatant was removed and discarded. The template was washed out three times by 200 µL of 10 % acetic acid and once by 200 µL of MilliQ.

2.3. Sample preparation

After washing of Mag-MIPs and Mag-NIPs by 20 mM TRIS (pH 8.5), 200 µL of sample was added and mixture was left shaking for 2 hours. After supernatant removal, Mag-MIPs and Mag-NIPs were washed three times with 200 µL of 20 mM TRIS (pH 8.5) and samples were analyzed by LA-ICP-MS.

2.4. LA-ICP-MS analysis

LA-ICP-MS experiments were performed using a setup consisting of the laser ablation system UP213 (NewWave Research, USA) emitting laser radiation with a wavelength of 213 nm. The ablated material was washed away using He (1.0 L·min^{-1}) from the ablation chamber (Supercell). Ar flow (0.6 L·min^{-1}) was admixed into a flow of helium with the sample aerosol behind the ablation cell. Hence, the total gas flow was 1.6 L·min^{-1}. This mixture was fed into a quadrupole ICP-(Q)MS spectrometer Agilent 7500CE (Agilent Technologies, Japan) equipped with a collision-reaction cell for suppressing possible polyatomic interferences. The CRC was utilized in collision mode with a He (99.000%) flow rate of 2mL·min^{-1}.

3. RESULTS AND DISCUSSION

The MIP-based separation procedure using MPs and following LA-ICP-MS analysis is schematically shown in **Figure 1.**

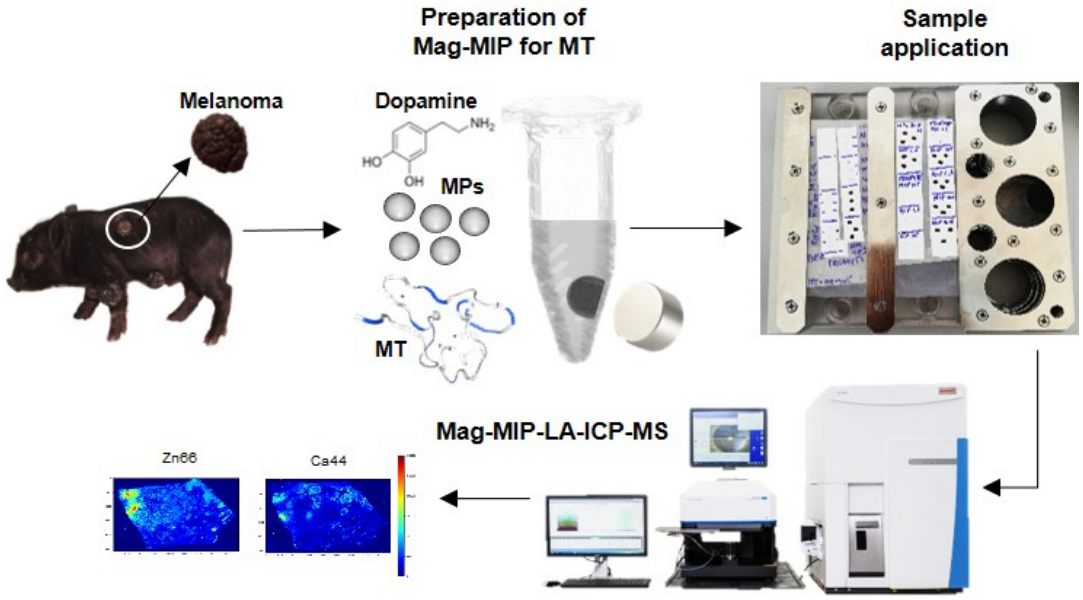

Figure 1 Schematic experimental workflow of Mag-MIP-LA-ICP-MS.

3.1. Metal detection in MT by LA-ICP-MS

Current analytical methods for MT detection include capillary electrophoresis, immunoassays, liquid chromatography, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and electrochemistry [10]. LA-ICP-MS is suitable for quantitative analysis of very low concentrations therefore; it was employed for quantitation of levels of metal ions present in MT.

In **Figure 2**, determined Zn levels are shown. The nonspecific sorption on polymeric layer without presence of cavities (NIP) was reasonably low and this background signal can be subtracted for quantification purposes.

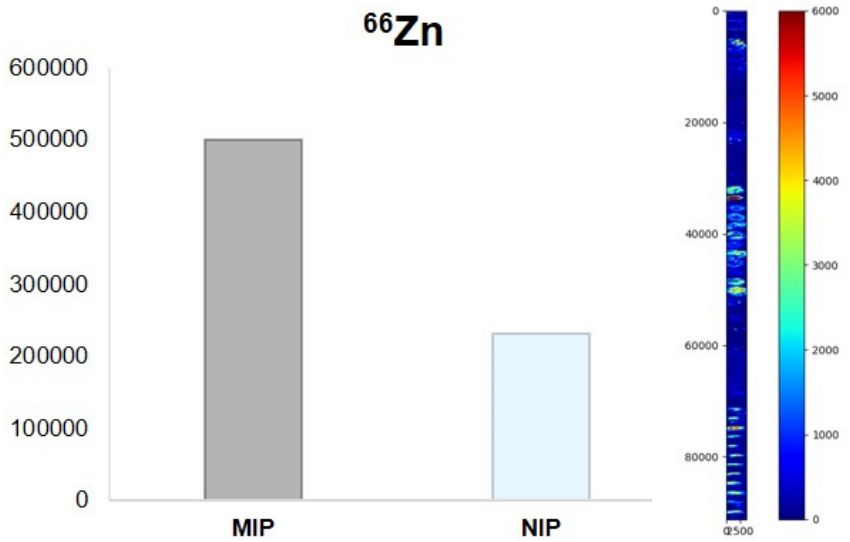

Figure 2 LA-ICP-MS analysis of ^{66}Zn levels after extraction of MT by Mag-MIP

3.2. Recognition of melanomas

For evaluation of melanoma, analysis of clinical parameters is important as well as identification of potential biomarkers. MT is highly probably one of such markers.

In this study, the melanomas and healthy skin of melanoma-bearing minipigs (MeLiMs) were tested using the magnetic MIPs specific for MT and it was found that levels of detected elements were significantly elevated in melanoma tissue compared to healthy skin (**Figure 3**).

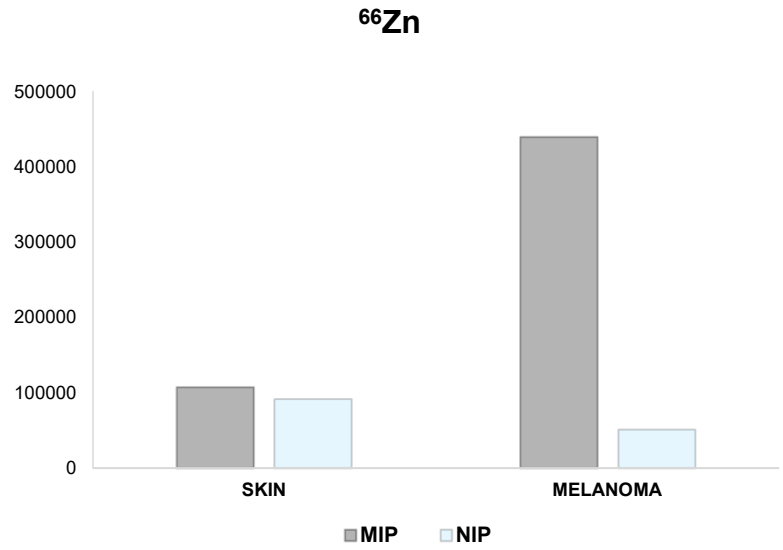

Figure 3 Levels of ^{66}Zn detected in MT isolated by Mag-MIP from healthy skin and melanoma of MeLiMs

4. CONCLUSION

In this work, power and variability of molecular imprinting technology in combination with high surface area of nanomaterials and magnetic properties of iron oxide creates an extremely effective tool for bioanalytical applications. Mag-MIP-LA-ICP-MS is a unique cross-border combination of molecular and elemental analysis. Mainly, the ^{66}Zn level was elevated in the majority of melanomas in comparison with healthy skin. This new approach enables the get information which is essential for further development of effective and timely diagnostic tools for melanoma detection.

ACKNOWLEDGEMENTS

The research was financially supported by AF-IGA20120-IP059.

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