Original Article

Improvement in Ionization Efficiency Using Metal Oxide Nanoparticles in Laser Desorption/Ionization Mass Spectrometry of a Cancer Drug

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Mass spectrometry imaging (MSI) without labeling has the potential for faster screening in drug development. Matrix-assisted laser desorption/ionization (MALDI) is typically used, but it has a large matrix size and uneven drug distribution. Surface-assisted laser desorption/ionization (SALDI) using nanoparticles (NPs) may overcome these issues. Here, the influence of NPs, solvent ratio, and order of dropping of NPs on SALDI-MSI of protoporphyrin IX (PpIX), a cancer drug, are reported. A solution of PpIX in a 50% aqueous solution of 50% acetonitrile at a concentration of $10\,\mu$M was used. The NPs include ZnO, Fe_3O_4, and four types of TiO_2. The NPs were fabricated by dissolving them on an aqueous 90% acetonitrile solution. Mass spectra were obtained with a time-of-flight mass spectrometer using a Nd:YAG laser at a 355-nm wavelength. The signal intensity using TiO_2 at a 0.5 mg/mL concentration in 50% acetonitrile was increased by 1.6-fold compared to that without TiO_2. Changing the solvent to 90% acetonitrile gave a uniform TiO_2 distribution and a 9-fold increase in the signal intensity for PpIX. Among the four types of TiO_2 with different particle sizes and crystal structures, TiO_2 with a smaller particle size and a rutile crystal structure produced the highest signal intensity. Forming a layer on top of the PpIX also resulted in an increased signal intensity. Hence, SALDI using TiO_2 provides effective ionization of the drug. In the future, we plan to investigate a spray method for the ionization of PpIX using TiO_2 for the MSI of various drugs.

INTRODUCTION

More than 10,000 candidates are generally required to develop one new drug. As such, the research and development period for a new drug is currently 10–15 years and the average cost is more than 800 million dollars.[1] Thus, enhancing the productivity of new drug development would be highly desirable. Screening is used to identify a target substance from a huge number of candidates in the early stages of drug development. High-throughput screening, which can comprehensively evaluate the efficacy, safety, and pharmacokinetics of a drug from the initial stage of development, is important.[2,3]

A faster screening method would clearly facilitate drug development. Currently, autoradiography is the most common method for drug screening,[4] but it has drawbacks such as increased costs due to labeling, long measurement times, and the need for the simultaneous measurements of metabolites. On the other hand, mass spectrometry imaging (MSI)[5,6] does not require labeling, which is advantageous in terms of cost and measurement time. In matrix-assisted laser desorption/ionization (MALDI), which is mainly used in MSI, aromatic compounds with an absorption peak in the ultraviolet region are widely used as ionization-assistant reagents.[7–9] It has been noted that, when the matrix is sprayed onto the sample, the crystal size of the matrix becomes large, and the drug distribution becomes uneven.

In recent years, surface-assisted laser desorption/ionization (SALDI) has been attracting interest as a potential solution to this problem.[10–12] In SALDI, metal oxide nanoparticles (NPs), which have absorption peaks in the ultraviolet region, are used as reagent for assisting the ionization process. In this method, the sample molecules adhere to the surface of the NPs and electrons or cations are then

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generated from the NPs electronically by excitation with a laser instead of the process in which the sample molecules and the matrix form mixed crystals. The crystals act like a matrix and may reduce the disorder of drug distribution. In addition, since matrix-derived ions are not detected in the small molecule region, the analysis of small molecule samples is facilitated.[13] To date, relatively high molecular weight lipids have been analyzed *via* SALDI, but there are few studies in which the types, particle sizes, concentrations, and surface conditions of NPs have been examined for MSI of drugs.[14] In this study, we report on the application of SALDI to drugs in an attempt to improve ionization efficiency.

EXPERIMENTAL

Time-of-flight mass spectrometer

All experiments were performed using a time-of-flight (TOF) mass spectrometer (Voyager DE-PRO; Applied Biosystems, Foster, CA, USA) equipped with a 355-nm third-harmonic Nd:YAG laser (GAIA II 30-T; Rayture Systems, Tokyo, Japan). The instrument parameters in the reflectron-mode of Voyager DE-PRO were set as follows: +20 kV acceleration voltage, +13.6 kV voltage for the extraction grid, 0 V for the guide wire, and 100 ns extraction delay time.

Scanning Electron Microscope

A scanning electron microscope (SEM, JCM-5700; JEOL, Tokyo, Japan) was used to observe the distribution of NPs in the sample spot.

Sample preparation

Protoporphyrin IX (PpIX, P8293; SIGMA-Aldrich, Tokyo, Japan), which has basic skeleton of hemoglobin, is also used as a cancer treatment drug and in photodynamic therapy (PDT). Although it is metabolized in normal tissue, it accumulates in cancer cells due to a metabolic abnormality.[15] In this study, PpIX is used as the cancer drug.

We investigated three types of metal oxide NPs: TiO_2, ZnO (APR5350; Wako, Osaka, Japan), Fe_3O_4 (MKCM9976; SIGMA-Aldrich). These NPs effectively ionize small molecules in samples.[16–18] In addition, three other types of TiO_2, with different sizes and crystal structures, were evaluated. The particle sizes and crystal structures of the four types of TiO_2 were 280 nm and rutile (CR-58; Ishihara Sangyo Kaisha, Osaka, Japan), 21 nm and anatase/rutile (718467-100G; SIGMA-Aldrich), 5–15 nm and rutile (US7050; US Research Nanomaterials, Houston, TX, USA), and 5 nm and anatase (7930DL; Skyspring Nanomaterials, Houston, TX, USA). The particle sizes, crystal structures and other information concerning the NPs can be found on the websites of purchasers mentioned above.

We examined two application methods of NPs with different matrix application orders. While dropping the matrix on the drug is a common approach in imaging, dropping the drug on the matrix may also be a feasible approach. Herein the NPs were applied under the sample so that the ionization efficiency was relatively high. After investigating sample preparation methods, the NPs were dropped onto the sample for MSI.

In this experiment, a metal plate (4347686; Applied Biosystems, Foster City, CA, USA) was used as the sample plate.

RESULTS AND DISCUSSION

Detection of PpIX using NPs

To investigate the efficiency of NPs for the ionization of PpIX, we measured PpIX in a mixture, which was prepared by mixing PpIX in water with 50% acetonitrile at a concentration of 10 μM. The NPs were dissolved in water with 50% acetonitrile at concentrations of 0, 0.1, 0.5, 1, and 5 mg/mL. In this experiment, the TiO_2 particles had a particle size of 21 nm and each NP was sonicated for 20 min. NPs with a volume of 1 μL were dropped onto the metal plate which was then dried in a vacuum. A mixture of pure PpIX in 1 μL of solution was dropped on the dried spot containing the NPs and mass spectra were obtained with a TOF mass spectrometer. A laser was randomly irradiated at 10 points for one spot and 100 pulses per point and the respective spectra were then averaged to obtain one mass spectrum.

Figure 1 shows typical mass spectra obtained from a mixture of pure PpIX with a volume of 1 μL using (a) no NPs, (b) TiO_2, (c) ZnO, and (d) Fe_3O_4 at a concentration of 0.5 mg/mL. Compared to the case without NPs, the signal intensity of the ion at *m/z* 563.3 increased when TiO_2 was used. Figure 2 shows the average signal intensities of the ions at *m/z* 563.3 obtained from a mixture of pure PpIX and different NP concentrations (0, 0.1, 0.5, 1, and 5 mg/mL). a concentration of 0 mg/mL of NPs denote the results obtained when only PpIX was examined in all figures. Using TiO_2 as reagent for assisting ionization enhanced the sensitivity of detection of the ions at *m/z* 563.3 by 1.6-fold compared to PpIX only. Although it has been reported that ZnO and Fe_3O_4 are effective in the ionization of small molecule samples and peptides,[16,17] the signal intensity of PpIX tended to decrease when these NPs were used. We conclude that the ionization efficiency of the sample differs depending on the chemical properties of the reagents being used in SALDI.

Investigation of the solvent ratio for TiO_2

TiO_2 was found to improve the ionization efficiency of PpIX among NPs. In SALDI, the ionization efficiency depends on the state of aggregation in the dried sample. Thus, we investigated the influence of the matrix solvent on ion-
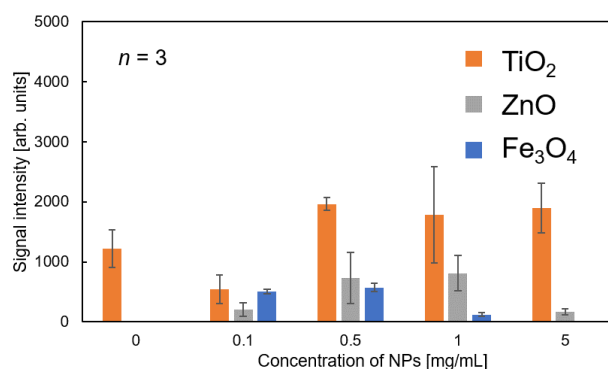

Fig. 1. Typical mass spectra obtained from a mixture of pure PpIX in a volume of 1 μL using (a) no NPs, (b) TiO_2, (c) ZnO, and (d) Fe_3O_4. PpIX was dropped on each NP. Particle size of TiO_2 is 21 nm.
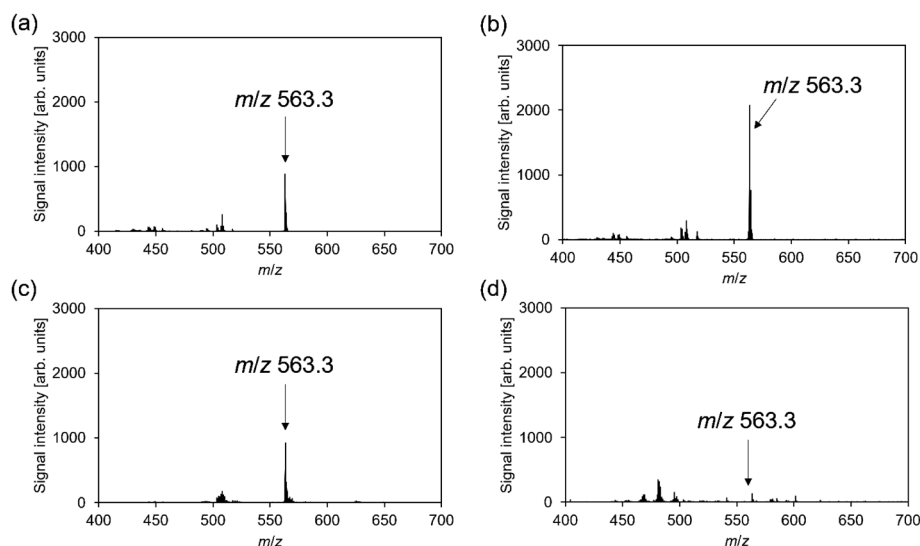
Fig. 2. Average signal intensities of the ions at m/z 563.3 obtained from a mixture of pure PpIX using NPs at concentrations of 0, 0.1, 0.5, 1, and 5 mg/mL. The particle size of TiO$_2$ was 21 nm.

ization efficiency and aggregation state.

A mixture of pure PpIX in water with 50% acetonitrile at a concentration of 10 μM was prepared. TiO$_2$ particles with a size of 21 nm were made by dissolving it in water with 10%, 50%, or 90% acetonitrile at a concentration of 0, 1, or 5 mg/mL. Increasing the proportion of acetonitrile above 90% caused the spots of the dropped TiO$_2$ to expand, making sample preparation difficult. Each TiO$_2$ sample was sonicated for 20 min. TiO$_2$ in a volume of 1 μL was dropped onto a metal plate and dried in a vacuum and PpIX was then dropped on the dried TiO$_2$ spot.

Figure 3 shows the average signal intensities for PpIX with TiO$_2$ at concentrations of 0, 1, and 5 mg/mL in water with 10%, 50%, or 90% acetonitrile. A 90% aqueous acetonitrile solution resulted in the highest signal intensity. The signal intensity of PpIX increased with increasing ratio of acetonitrile. The signal intensity was about 12.7-fold higher than that without TiO$_2$.

Figure 4 shows SEM images obtained from dropping spots of TiO$_2$ at a concentration of 1 mg/mL dissolved in water with 10% or 90% acetonitrile. The NPs were more uniformly distributed in the spots in the case of a 90% acetonitrile aqueous solution compared to a 10% acetonitrile aqueous solution. Since acetonitrile is more volatile than water, a homogeneous layer of TiO$_2$ would be formed. The data shown in Figs. 3 and 4 indicate that when the proportion of acetonitrile is increased, the distribution of NPs becomes uniform and the signal intensity of PpIX increases because it is less affected by aggregation.

Comparison of ionization efficiency using four types of TiO$_2$

In this experiment, we investigated the sensitivity of detection of PpIX using four types of TiO$_2$. A mixture was prepared of pure PpIX in water with 50% acetonitrile at a concentration of 10 μM. The four types were prepared by dissolving TiO$_2$ in water with 90% acetonitrile at concentrations of 0, 0.1, 0.5, 1, 5, and 10 mg/mL. Each TiO$_2$ sample was sonicated for 20 min and a volume of 1 μL was then dropped onto a metal plate and dried in a vacuum, after which, the PpIX was dropped on the dried spot of TiO$_2$.
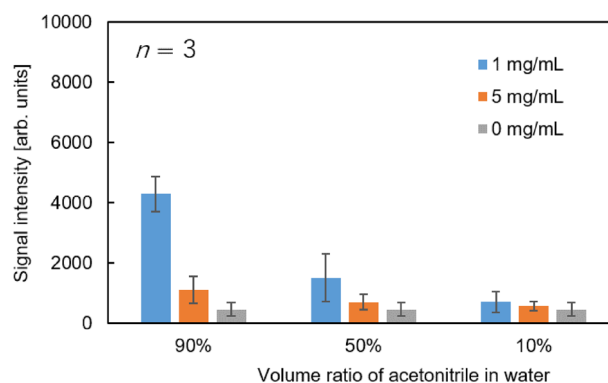

Fig. 3. Average signal intensities of ions at m/z 563.3 with TiO$_2$ at concentrations of 0, 1, and 5 mg/mL using 10%, 50%, or 90% acetonitrile as the solvent. PpIX was dropped on the TiO$_2$. The particle size of TiO$_2$ was 21 nm.

Figure 5 shows the average signal intensities of PpIX when the four types of TiO$_2$ were used. The highest signal intensity was obtained when 5–15 nm TiO$_2$ was used. The signal intensity was about 9.0-fold higher than that obtained from PpIX only. A peak corresponding to protonated PpIX was not obtained when 280-nm TiO$_2$ was used at concentrations of 5 and 10 mg/mL. This suggests that the signal intensity decreases with increasing particle size when high concentrations of TiO$_2$ are used. The signal intensity was higher when 5–15-nm TiO$_2$ was used compared to the use of 5-nm TiO$_2$. This difference can be attributed to the crystal structure. TiO$_2$ with a particle size of 5–15 nm has a rutile crystal structure. Generally, in SALDI, a rapid temperature increase in the pulse laser irradiation greatly affects the efficiency of sample ionization. The UV absorption efficiency of rutile-type TiO$_2$ is higher than that of the anatase-type.[19] Therefore, the signal intensity obtained from PpIX using 5–15 nm TiO$_2$, which has a rutile-type crystal structure and a small particle size, was the highest.

Comparison of ionization efficiency by order of dropping of NPs

In the above experiments, PpIX was dropped onto NPs.
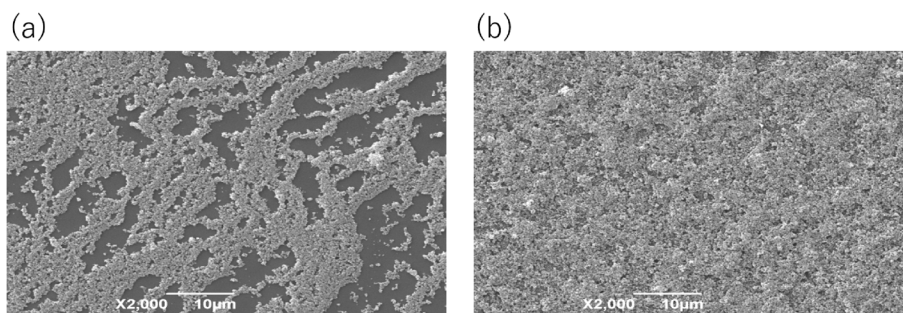
(a)

(b)

Fig. 4.   SEM images obtained from dropping spots of TiO_2 dissolved in water with (a) 10% and (b) 90% acetonitrile at a concentration of 1 mg/mL.
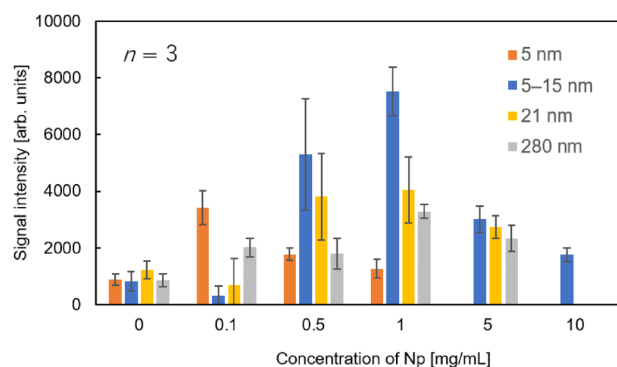

Fig. 5.   Average signal intensities of ions at m/z 563.3 using four types of TiO_2 at concentrations of 0–10 mg/mL. The PpIX was dropped on the TiO_2.
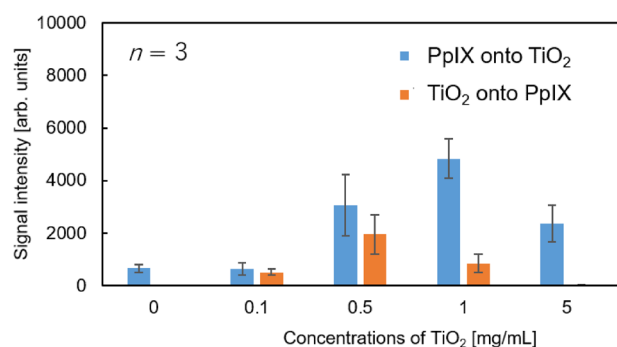

Fig. 6.   Average signal intensities of ions at m/z 563.3 with TiO_2 at a concentration of 1 mg/mL using 90% acetonitrile as the solvent in two dropping orders of TiO_2. The particle size of TiO_2 is 21 nm.

In MSI, it is necessary to form a layer of NPs on the measurement sample. The effect of the order of dropping on the detection sensitivity was investigated.

A mixture of pure PpIX was prepared in water with 50% acetonitrile at a concentration of 10 μM. TiO_2 NPs with a particle size of 21 nm were prepared by dissolving TiO_2 in water with 90% acetonitrile at a concentration of 0, 0.1, 0.5, 1, or 5 mg/mL. Each TiO_2 sample was sonicated for 20 min. PpIX or TiO_2 with a volume of 1 μL was dropped onto a metal plate, dried in a vacuum and TiO_2 or PpIX was then dropped on the dried spot.

Figure 6 shows the average signal intensities for PpIX with TiO_2 at concentrations of 0, 0.1, 0.5, 1, and 5 mg/mL using 90% acetonitrile as the solvent for the two dropping orders. When a layer of NPs was formed on top of the PpIX, the signal intensity was reduced. This tendency was more pronounced as the concentration of TiO_2 was increased. In SALDI, NPs absorb energy from the laser irradiation and then transfer it efficiently to the analyte. When TiO_2 is dropped onto the sample, the energy is absorbed by the layer of TiO_2 on the surface of the sample, and it is generally assumed that the efficiency of energy transfer to PpIX decreases. Further, regardless of the dropping order, when the concentration of TiO_2 becomes too high, the signal intensity is lowered because the NPs undergo aggregation. The signal intensity was the highest when TiO_2 was dropped on PpIX at a concentration of 0.5 mg/mL and was about 3-fold higher than the value without PpIX. The spot diameter of TiO_2 was about 1 mm. The estimated suitable density of TiO_2 for ionization of PpIX was about 0.64 μg/mm^2.

For MSI, an ionization-assisting reagent was applied on the sample using various methods.[20–22] In future work, we plan to investigate the spray method for the ionization of PpIX using TiO_2.

CONCLUSION

For SALDI-MSI of a cancer drug using NPs, we investigated different factors: the type of NPs used, solvent ratio, and dropping order for the ionization of pure PpIX. In initial experiments, we examined the ionization efficiencies of PpIX using TiO_2, ZnO, and Fe_3O_4, which are appropriate for the ionization of samples containing small molecule. The signal intensity using TiO_2 with 50% acetonitrile at a concentration of 0.5 mg/mL was 1.6-fold higher than that without TiO_2. In addition, changing the solvent for the TiO_2 to 90% acetonitrile resulted in the uniform distribution of TiO_2, and the signal intensity of PpIX was increased 9-fold. The signal intensity of PpIX tended to decrease when ZnO and Fe_3O_4 were used in this experiment. These NPs were found to be unsuitable for the ionization of PpIX. We next investigated four types of TiO_2 samples with different particle sizes and crystal structures. The highest signal intensity for PpIX was obtained using TiO_2 with a 5–15 nm particle size and a rutile crystal structure. The signal intensities of PpIX were compared for different orders of dropping of TiO_2 for MSI. When a layer of NPs was formed on top of the PpIX, the ionization efficiency decreased. However, the signal intensity was about 3-fold higher than that for PpIX only. These results demonstrate that the use of TiO_2 in SALDI can be used to effectively measure PpIX. In the future, we plan to investigate the spray method for the ionization of PpIX using TiO_2 for the MSI of various drugs.

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