MALDI-Ion Mobility Mass Spectrometry Imaging for Paclitaxel Nanomedcine Distribution In Solid Tumor Tissue

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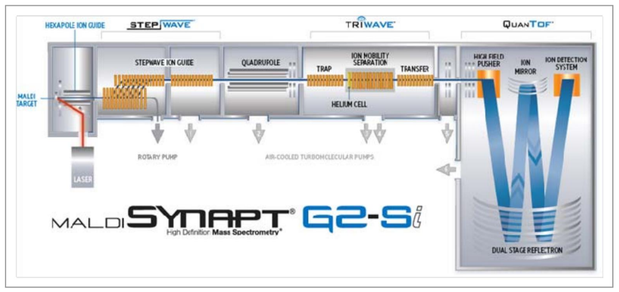
Introduction

In drug discovery and development, it is important to understand the biodistribution and accumulation of drugs in tissue since drug efficacy strongly depends on the presence of the drug substance at the target site. MALDI mass spectrometry imaging (MSI) has become a powerful tool for the detection and localization of drugs. The combination of MALDI and ion mobility enable the mass and time selected ion images. Here, we report a specific approach to explore the heterogeneous distribution of paclitaxel (PTX) in solid tumor by MALDI-ion mobility MSI using commercially available TiO_2 nanoparticles as the matrix. Four clinically approved paclitaxel nanoparticle formulations were studied. In addition, the MSI results were coregeistered with immunohistochemical staining to evaluate the location of drug on tissue.

Method

Instruments and software

A MALDI-ion mobility mass spectrometer Synapt G2-Si Qtof (Waters Corporation, USA) was used for imaging of the tissue samples. The ion source has a 355 nm Nd:YAC laser with a 100-2500 Hz repetition rate, controlled by the software MassLynx V4.2 from Waters. MS spectra were acquired with an automatic scan rate under sensitivity mode with positive or negative ionization modes. The MALDI source settings were set with 0.5 scans per pixel, 1000 or 2000 HZ laser firing rate and 400 laser energy. The instrument was mass calibrated with Red Phosphine before analysis to have less than 2 ppm root mean square error on accuracy. HDImaging software V1.4 from Waters was used to process and visualize ions distribution inside the tissue sections.



sample preparation

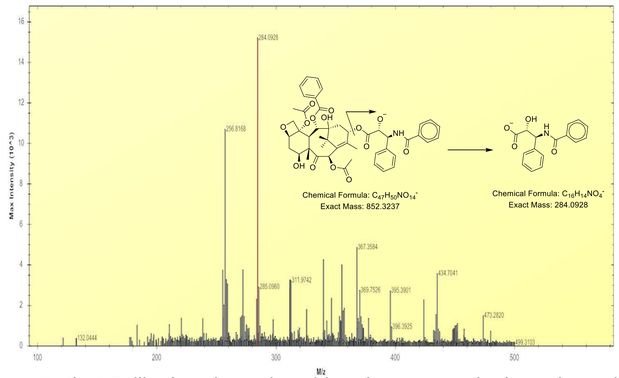
Xenograft tumors were harvested 4 hours after dosing drugs, directly frozen in liquid nitrogen for further analysis. Tissue cryosections of 15 µm thickness were cut and mounted onto precooled glass plate or metal imaging plate. For MALDI drug imaging, tissue sections on plate were dried in a vacuum dryer at room temperature. Then the slices were sprayed with TiO_2 matrix suspension including 200 ng/mL reference compound D5-paclitxel.

Results

MS imaging method development on steel MALDI plate

For identification and optimization of the paclitaxel peaks, multiple commonly used MALDI matrices have been tested for ionization of paclitaxel on steel plate.

Figure 1. MS spectra of PTX. In negative mode using TiO_2 as the matrix, the in source fragment ions at m/z 284.0928 is clearly predominant.



Both 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid show lower ionization efficiency to generate parent, and adduct ions with Na^+ or K^+ in MS experiment. In addition, high matrix ion interference and background noise were found during test. When using TiO_2 nanoparticle as the matrix, paclitaxel and reference D5-paclitaxel were efficiently ionized under negative mode to form in source fragment ions at m/z 284.092 and m/z 289.127 respectively, corresponding to the amide-acyl group on the side chain. Both fragment ion peaks can be enhanced by ion mobility separation compared to Tof mode.

Figure 2. ESI TOF MS/MS scan of PTX $[M-H]^-$. By infusion in negative mode, the fragment ion at m/z 284.09 is observed with low intensity.

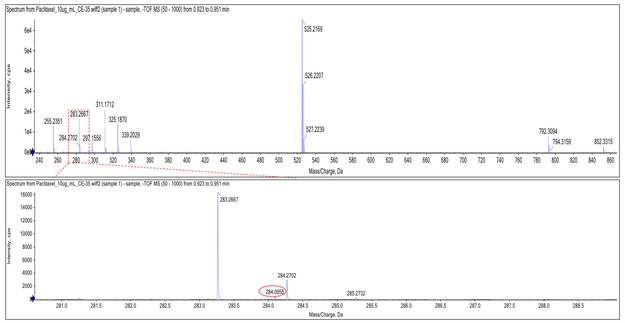
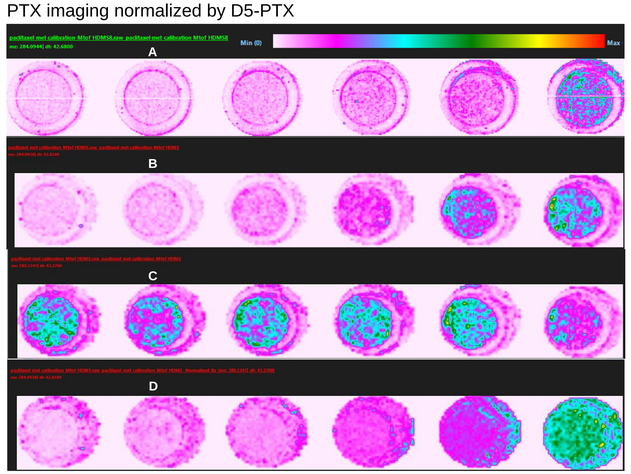


Figure 3. Increasing amounts of PTX (0.9, 2.25, 4.5, 9, 18, 45 pmol) were co-spotted with a constant amount of D5-PTX (18 pmol) on steel MALDI plate and TiO_2 as the matrix. **A**) PTX with space resolution 50 µm. **B**) PTX with space resolution 100 µm. **C**) D5-PTX with space resolution 100 µm. **D**) PTX imaging normalized by D5-PTX



MS ion image of PTX (side chain m/z 284.092) in Tumor

MMTV-PyMT Transgenic spontaneous breast cancer Mice were treated with four formulations of paclitaxel (Taxol, Abraxan, Paclical, or Genexol-PM, 10 mg/kg) by IV. Tumor section were used for MS imaging and CD31 staining.

Figure 4. **A)** Tumor tissue section were mounted on glass plate, then put on MALDI plate holder. **B)** The structure of Paclitaxel, D5-Paclitaxel and nano formulations. **C)** Imaging of 9 pmol PTX on blank tissue at 75 µm laser spot.

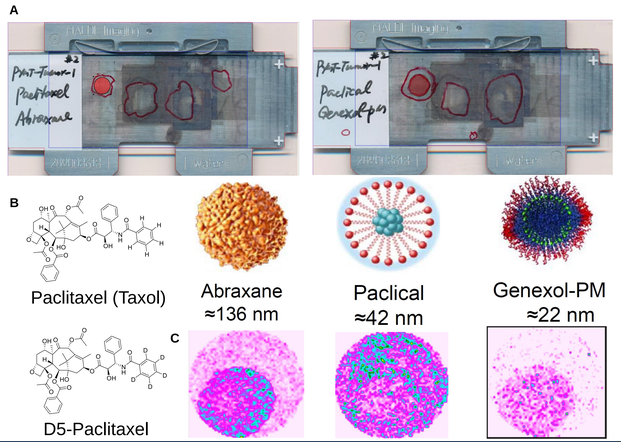
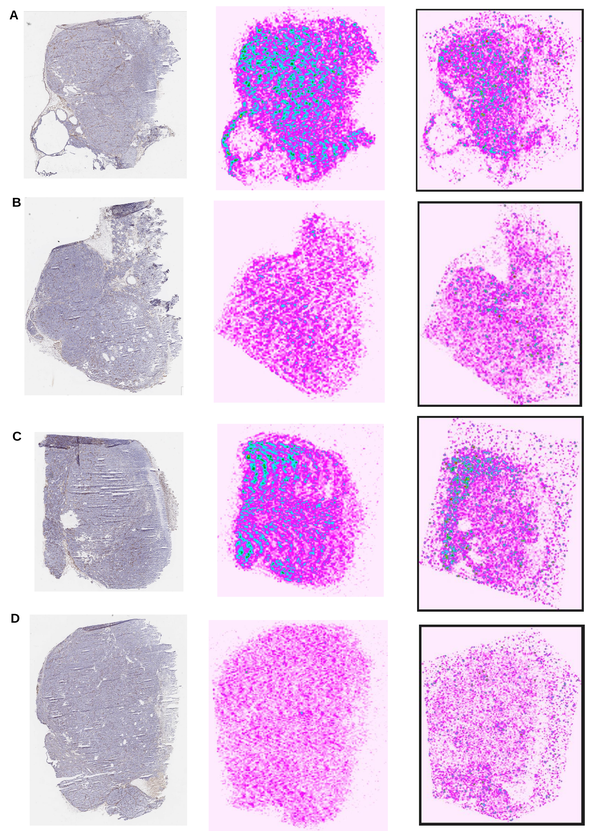


Figure 5. **CD31 staining and PTX distribution in tumor from MMTV-PyMT Transgenic spontaneous breast cancer mice, then the images were normalized by D5-PTX. A)** Treated with Taxol **B)** Treated with Abraxane **C)** Treated with Paclical **D)** Treated with Genexol-PM.



Conclusion

The images received from CD31 staining were overlayed with the MALDI imaging data, which allows the correlation of nano medicine drug distribution and immunostaining on tissue. The mass imaging and CD31 staining results show strong drug signals located close to vessels. Tumor regions with more vessel has higher amount of drug substance. This study can be used to investigate the distribution of paclitaxel nanomedicine in tumors and help to develop new formulation to increase the efficacy of drugs.

Reference: 1) PLoS One. 2013 Aug 26;8(8). **2)** Sci Rep. 2016 Dec 21;6:39284.**3)** Sci Rep. 2016 Nov 14;6:37027.

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