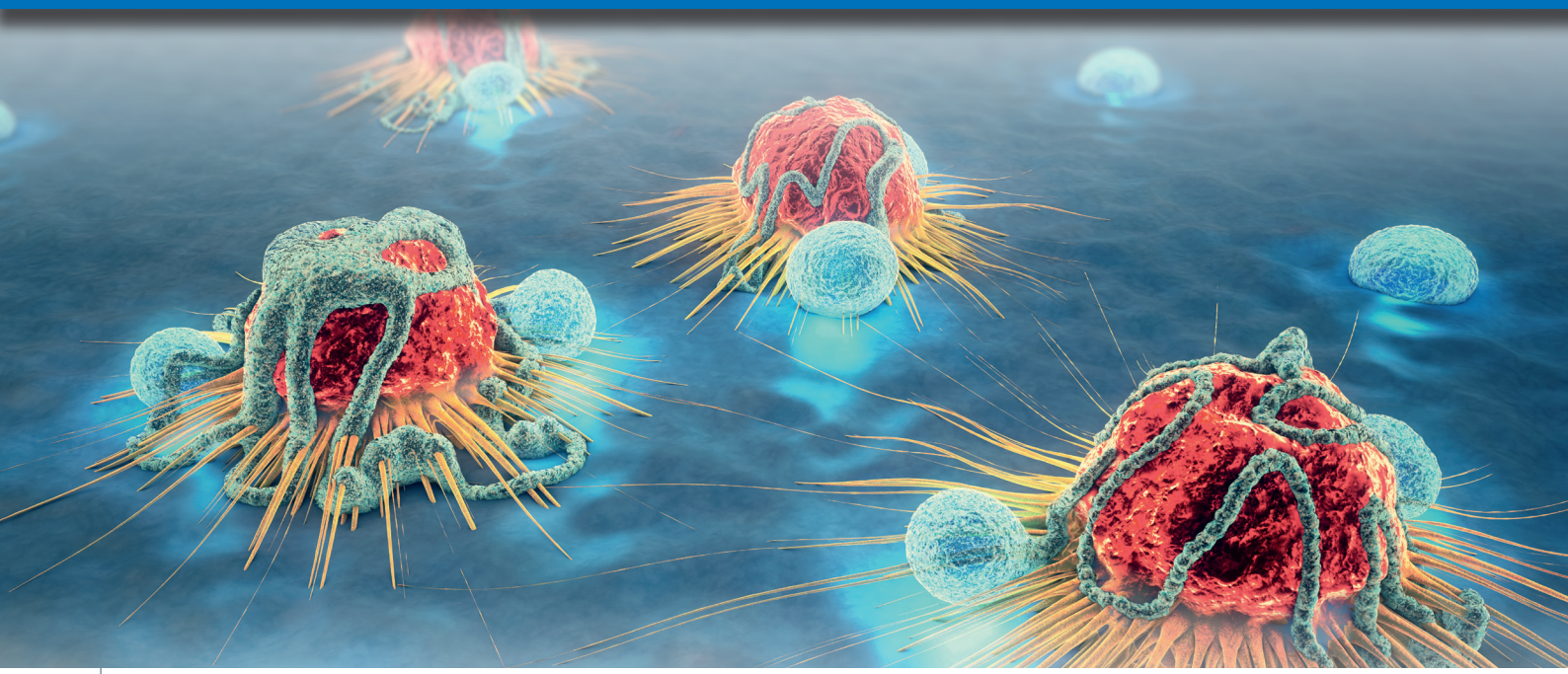

Small Drug Molecules MALDI-MS Imaging on Colorectal Tumor Organoids

The ultrafleXtreme TOF/TOF was used to image colorectal tumor organoid (CTO), treated with chemotherapeutic drug irinotecan. The ultrafleXtreme was able to detect the drug molecule and its metabolites, SN-38 and SN-38G, and distinguish the effects of irinotecan treatment time and concentration on CTOs, showing differentiated drug penetration at 6 and 24 h, and at 20 and 40 µM.

After analyzing in fleximaging and SCiLS Lab, the data demonstrated a spatial and chronological co-relationship between irinotecan and its metabolites that is highly relevant to elucidating the efficacy of the drug in tumor models.

Introduction

The efficacy of chemotherapy is highly dependent on the individual drug penetration into the target tumors, its uptake by cancerous cells, and the metabolism of the drug into its active form [1]. The effective dosage of therapeutics encountered by cells within a tumor is highly variable, largely due to the heterogeneity of its vascular structures [1,2].

The problem of drug delivery into tumors remains a challenge, affecting treatment regimen and the severity of side effects [3,4].

Visualizing drug penetration and uptake requires resolving the molecules spatially within tumors. Optical techniques such as microscopy and fluorescence can track metabolic activity in organoids but lack the ability to directly image drug molecules and its metabolic products [5-8].

This application note demonstrates the capability of MALDI-TOF mass spectrometry imaging to detect and spatially resolve small

Keywords: Irinotecan, mass spectrometry imaging, chemotherapy, colorectal tumor, organoids, small molecule, ultrafleXtreme, data analysis

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molecules, particularly irinotecan penetration into patient derived CTO, its metabolites, the active SN-38 and the inactive SN-38G. This note also covers the data analysis of the metabolism and permeability of irinotecan in CTOs based on drug concentration and treatment duration.

Experimental

The detailed CTO growth protocol and irinotecan treatment regimen can be found in the article by Liu and co-workers [9]. The CTOs sizes range from 50 to 500 µm. For the time dependent study, CTOs were treated with 20.6 µM irinotecan in DMSO for 6 and 24 h (n=5). In the concentration dependent study, separate CTOs were treated with 20 or 40 µM (n = 3) irinotecan for 72 h. CTOs suspended in blank DMSO acted as controls.

After treatment, CTOs were embedded in gelatin and cryosectioned at 12 µm thickness using the Leica CM1850 cryostat (Leica Microsystems, Wetzlar, Germany). Since the CTOs were not visible to the naked eye, H&E or immunofluorescence staining was used on the slices embedded on slides to detect them. Once CTOs were detected, consecutive slices were then slated for MS imaging.

Using TM Sprayer the slide was first sprayed with the internal standard (IS), irinotecan-d10 HCl (Santa Cruz Biotechnology, Santa Cruz, CA, USA), before being stored in a dessicator overnight under vacuum. The slide was then sprayed with matrix super-DHB (Sigma, St. Louis, MO, USA). Parameters for both spray preparations are listed in Table 1.

MS-images were obtained on the ultrafleXtreme in reflector, positive ion mode in the mass range of 200 to 1000 m/z at 1000 Hz. Spatial resolution was 35 µm using small

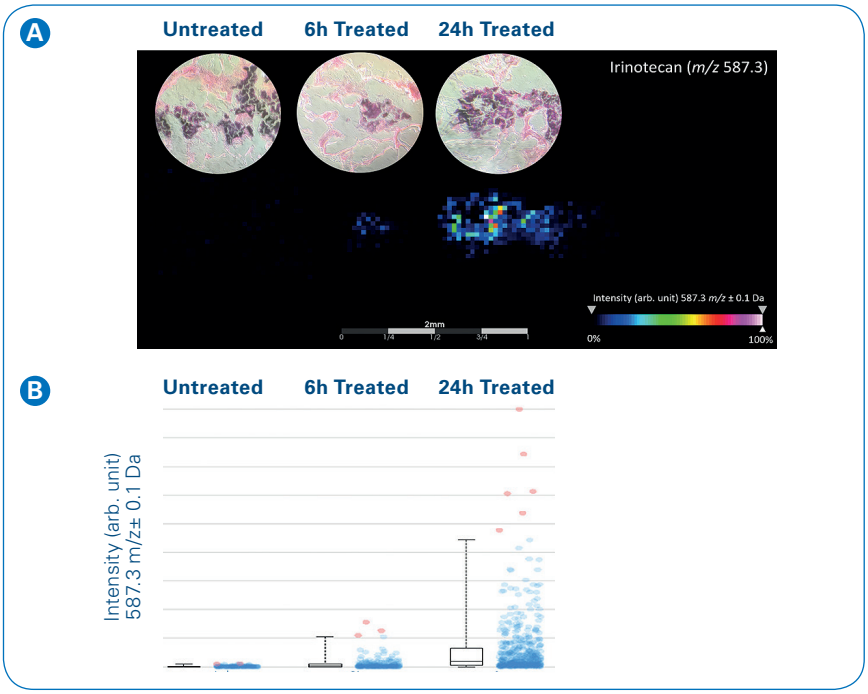

Figure 1: MALDI-MSI ion intensity maps and H&E stained images (A), and intensity box-whisker plots (B) of organoids treated with 20.6 µM irinotecan for 0, 6, and 24 h. The box-whisker plots show a median intensity. Blue dots represent the spectra in which intensities of the given m/z interval is between the lower and upper quantiles, and red dots represent outliers.

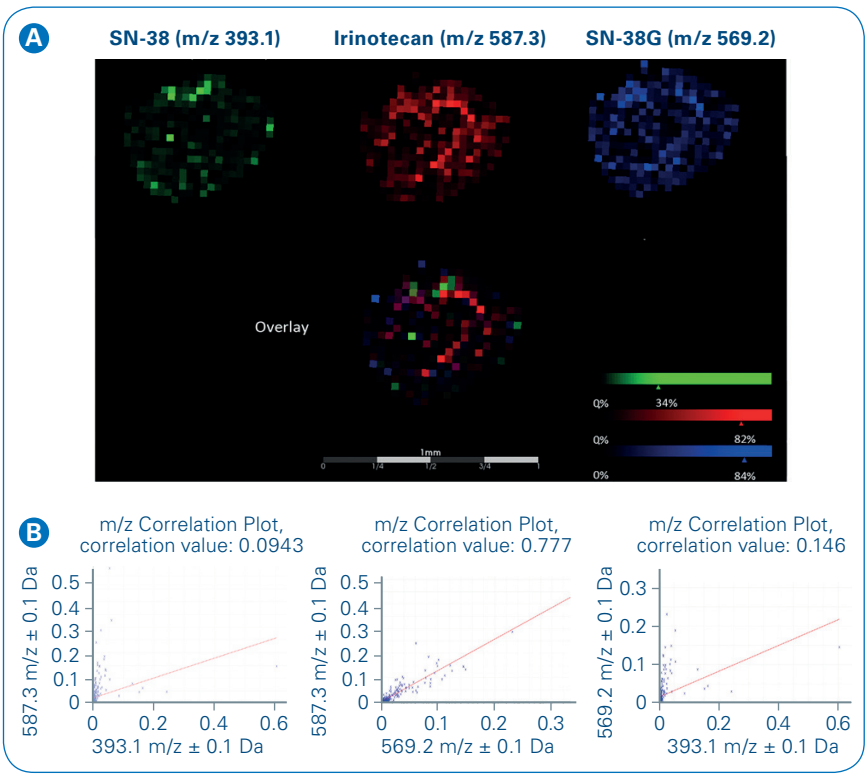

Figure 2: Localization of irinotecan and its metabolites in a 24 h treated CTO. (A) MALDI-MSI ion images of irinotecan (m/z 587.3), SN-38 (m/z 393.1), and SN-38G (m/z 569.2). (B) Correlation analysis to evaluate co-localization of irinotecan and its metabolites in the CTO. The left plot shows the correlation of irinotecan (m/z 587.3) and SN-38 (m/z 393.1). The middle plot shows the correlation of irinotecan (m/z 587.3) and SN-38G (m/z 569.2). The right plot shows the correlation of SN-38G (m/z 569.2) and SN-38 (m/z 393.1).

laser focus setting. Calibration was done externally with Bruker peptide standard spotted on unsprayed regions of the glass slide away from CTO slices. Spatial segmentation by SCiLS Lab was used to distinguish gelatin from CTO regions, allowing CTOs to be regrouped as separate regions for further analysis.

Results and Discussion

Irinotecan (m/z 587.3) was detected at high average ion intensity, with distinctively deeper penetration into the CTO at 24 h treatment than at 6 h (Figure 1). The metabolites SN-38 (m/z 393.1) and SN-38G (m/z 569.2) were also detected. Interestingly, correlation analysis showed higher spatial correlation between irinotecan and the inactive SN-38G than with SN-38 (Figure 2). The results suggest that the variable metabolism rate of irinotecan could be due to the diversity of the cell types in the CTOs.

Relative quantification using IS showed that a higher concentration of irinotecan was associated with an increased uptake and metabolism within the cells. However, SN-38 abundance scaled at a lower rate than the increase in irinotecan concentration, suggesting limited metabolic rate of irinotecan to SN-38.

Table 1: Spray parameters for internal standard and matrix.

Spray Type	Internal Standard (Irinotecan-d10)	Matrix (Super-DHB)
Solvent	H_2O	50% ACN, 0.2% TFA (TA50)
Temp (°C)	30	70
Number of passes	4	8
Concentration	50 µM	10 mg/mL
Flow rate (mL/min)	0.03	0.1
Track velocity (mm/min)	1000	1000
Track spacing (mm)	2	2
Drying time (s)	30	30
Gas pressure (psi)	10	10

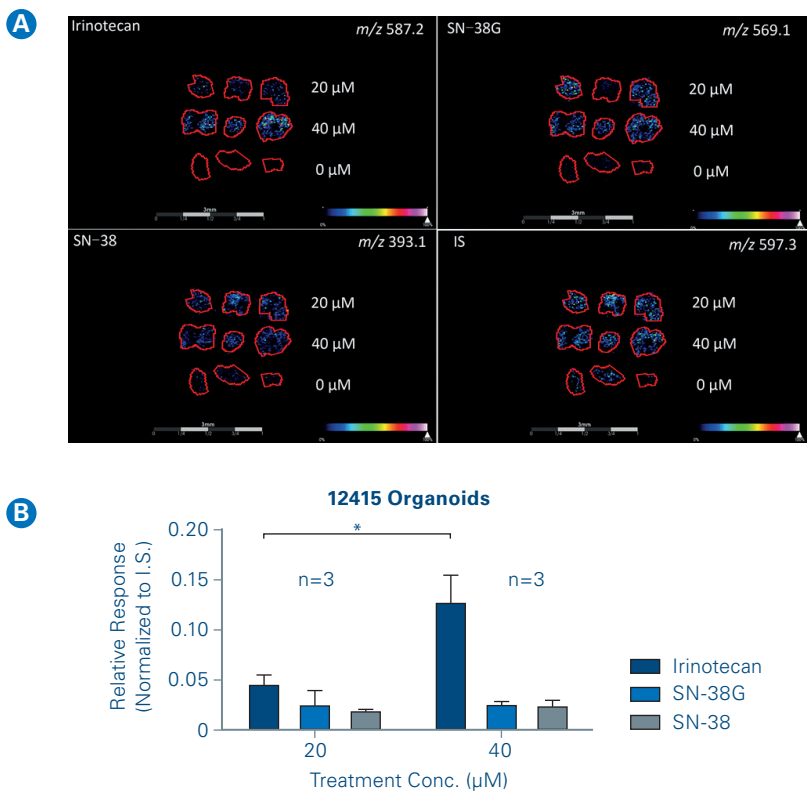

Figure 3: MALDI-MSI results of irinotecan treated CTOs at different concentrations for 72 h. (A) Ion density maps of irinotecan (m/z 587.2), SN-38 (m/z 393.1), SN-38G (m/z 569.1), and the IS (m/z 597.3) in 0, 20, and 40 µM drug treated CTOs. (B) Relative quantification of irinotecan, SN-38, and SN-38G in treated CTOs.

Conclusion

- Bruker's ultrafleXtreme MALDI-TOF imaging system was able to give spatial information relevant to irinotecan penetration and uptake within the CTOs based on concentration and treatment time of irinotecan.
- MALDI Imaging was also able to distinguish irinotecan and its metabolites. Analysis done in FlexImaging and SCiLS Lab demonstrates co-relationships between ions spatial distribution and their relative concentration, data which are highly relevant to discerning irinotecan's efficacy in CTO complex structure and heterogenous cell types.

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