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Preparation of a Mimetic Tissue Model for Mass Spectrometry Imaging Experiments.

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We use this protocol and it's working

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Disclaimer

This protocol is adapted from previously published methods describing the preparation of mimetic tissue models for imaging mass spectrometry, ([Revised Preparation of a Mimetic Tissue Model for Quantitative Imaging Mass Spectrometry, Full article: Quantification and Assessment of Detection Capability in Imaging Mass Spectrometry Using a Revised Mimetic Tissue Model](#)). The protocol has been modified to reflect specific workflows, and optimisations implemented within the Mass Spectrometry Core, including simplified mould preparation, and integration into routine quality control and method development pipelines. The authors acknowledge the original sources of this protocol.

Abstract

This protocol describes the preparation of a mimetic tissue model for mass spectrometry imaging (MSI) applications, suitable for both MALDI-MSI and DESI-MSI workflows. The method involves cryogenic homogenisation of biological tissue, controlled spiking of analyte standards or internal standards, and moulding into a frozen plug that closely mimics the physicochemical and ionisation properties of native tissue.

The resulting mimetic tissue model enables optimisation of matrix selection, matrix application parameters, and instrument settings, and supports the generation of calibration curves for qualitative and quantitative MSI analyses. When implemented correctly, this protocol produces homogeneous, reproducible tissue standards that can be used as quality control samples to assess analytical performance, signal stability, and inter- and intra-batch variability across extended MSI experiments.

Guidelines

- This protocol should be performed only by trained users familiar with mass spectrometry imaging and tissue handling procedures.
- All work must be carried out in accordance with institutional standard operating procedures, risk assessments, and local biosafety regulations.
- Ethical approval must be obtained prior to the use of animal or human tissue, and all samples must be appropriately documented.
- The tissue matrix used for the mimetic model should match the biological samples as closely as possible to ensure representative ionisation and extraction behaviour.
- Samples should be maintained at low temperature throughout homogenisation, spiking, and moulding to preserve tissue integrity and analyte stability.
- The total volume of spiked standards should be minimised (typically $\leq 2\text{--}3\%$ v/v of the homogenate) to maintain tissue morphology and homogeneity.
- Thorough mixing after spiking is essential to ensure even analyte distribution within the homogenate.
- Positive displacement pipettes should be used for accurate volumetric transfer and to minimise air bubble formation.
- Mimetic tissue models should be prepared, stored, and sectioned under consistent conditions to ensure reproducibility across experiments.
- These models are intended for method optimisation, calibration, and quality control and should be applied within the same analytical run as the biological samples of interest.

Materials

****Equipment:****

- Bead homogeniser with cryo-cooling system (Omni Bead Ruptor Elite)
- Centrifuge (set at 4 °C)
- Analytical balance
- Vortex mixer
- Positive displacement pipette (Microman M250, Gilson)

****Consumables and Chemicals:****

- Flash frozen Tissue (e.g. liver, brain, kidney or other relevant matrix)
- 2 mL homogenising tube with 2.8 mm Ceramic beads (Fisher Scientific, part no. 15565799)
- 1 mL syringe (for mould preparation)
- Dry ice
- Wet ice
- Analyte standard solution
- Hydrogel (for block preparation) – Prepare hydrogel a day before, follow hydrogel protocol

Safety warnings

- ❗
 - Biological tissues may present a biohazard risk. Handle all samples using appropriate personal protective equipment and in accordance with institutional biosafety guidelines.
 - Dry ice and frozen materials can cause cold burns. .
 - Bead-based tissue homogenisers generate mechanical force and noise. Ensure that lids and safety interlocks are fully secured before operation and follow manufacturer safety instructions.
 - Avoid high-speed or prolonged centrifugation of tissue homogenates, as this may cause phase separation and compromise analyte distribution.
 - Introducing air bubbles during homogenate transfer can lead to voids in the mimetic tissue model and poor sectioning quality. Take care during pipetting and dispensing steps.
 - Repeated freeze–thaw cycles may degrade analytes and affect quantitative performance. Aliquot tissue homogenates where possible and minimise thawing events.
 - Dispose of biological waste, solvents, and sharps in accordance with institutional and local environmental health and safety regulations.

Ethics statement

All experiments involving animal tissue must be conducted in accordance with internationally accepted standards for the care and use of laboratory animals. Prior ethical approval must be obtained from an Institutional Animal Care and Use Committee (IACUC) or an equivalent institutional or national ethics committee before performing these experiments. All procedures should comply with relevant legislation, institutional policies, and approved protocols governing the use of animal-derived materials.

Before start

- Trained staff, students, and visiting researchers must have read and signed all relevant risk assessment document(s) associated with this SOP before performing the procedure.
- Users must be trained to operate the Omni Bead Ruptor Elite, Cryo Cooling Unit, and EKOM DK50 2VS/M Air Compressor within the Mass Spectrometry Core.
- Prepare and pre-cool all consumables, including homogenisation tubes, beads, and mould components, on dry ice before beginning tissue processing.
- Prepare the hydrogel embedding medium in advance (typically the day before) according to the relevant protocol and store under appropriate conditions.
- Verify that analyte standards are prepared at appropriate stock concentrations using water-miscible solvents and are compatible with the intended MSI workflow.
- Review the full protocol and calculations in advance to minimise handling time at ambient temperature and reduce the risk of sample warming.



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Standard Preparation and Calculation for Homogenate Spiking

30m

- 3 The total volume of the spiked standard(s) should be kept below 2-3% (v/v) compared to the homogenate volume. The solvent used to dissolve the standard should be water miscible to ensure efficient mixing with the homogenized tissue.
- 4 To determine the appropriate concentration for spiking, first perform a MALDI experiment using a dilution series of the analyte standard (e.g. 1 mg/mL, 500 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 20 µg/mL, 10 µg/mL).

Mix each dilution 1:1 (v/v) with MALDI matrix and spot 1 µL onto a steel target plate. Acquire MALDI spectra and determine the limit of detection (LoD).

Use one concentration above the LoD for spiking the tissue homogenate.

Example: When 10 µg/mL standard mixed 1:1 with matrix and spotted on steel plate, the dried MALDI spot concentration would be 5 µg/mL. If 5 µg/mL represents the LoD, then a target concentration 10 µg/mL = 10 µg/g tissue should be used for the mimetic tissue model.

- 5 **Calculation for 10 µg/g Analyte Concentration in 300 mg Tissue:**



- Take 10 µg of each analyte (10 µL of a 1 mg/mL stock) into a vial and dry under nitrogen.
- Reconstitute in 20 µL solvent to prepare a 500 µg/mL stock solution.
- Add 6 µL of the 500 µg/mL stock solution to 300 mg tissue homogenate.

This results in:

- $6\ \mu\text{L} \times 500\ \mu\text{g/mL} = 3\ \mu\text{g analyte}$
- $3\ \mu\text{g analyte in 300 mg tissue} = 10\ \mu\text{g/g tissue}$

***Remember: maximum 6µL can be added in 300 mg tissue.**

- 6 Add the calculated volume of standard to the homogenate and mix thoroughly.

Once the standard has been added, homogenise further at 4 m/s for ~30 seconds 2-3 times. This mixing step is critical to ensure even distribution of the standard throughout the homogenate.

Preparation of the Mould

15m

- 7 **Prepare the mould using a modified 1 mL syringe.**

- 7.1 Trim the syringe barrel and plunger seal. Cut off the luer tip using a blade or tubing cutter.
- 7.2 Trim the conical tip of the plunger seal to prevent conical distortion of the mimetic tissue model.
- 7.3 Pull back the plunger slightly and place the syringe vertically in dry ice, ensuring it is sufficiently submerged to keep the mould cold during rapid freezing of the tissue homogenate.

Preparation of Tissue Homogenate

30m

- 8
 - Pre-cool 2 mL reinforced tubes containing 2.8 mm ceramic beads on dry ice.
 - Keep tubes frozen on dry ice until homogenise.
- 9
 - Cut tissue into small pieces suitable for the homogenising tube.
 - Weigh approximately 500 mg of tissue into the pre-cooled tube. Do not overfill; only fill about 3/4 of the tube to allow space for beads and efficient homogenisation. **Keep frozen on dry ice.**



- 10 Follow **Operation Protocol OP_MS_04**, latest Version (**Operation of the Mass Spectrometry Core Omni Bead Ruptor Elite and Cryo Cooling Unit, QMRI**) for tissue homogenisation.
- 11
 - Homogenise at 4 °C using the Bead Ruptor Elite at 4 m/s for 30 s (2-3 cycles).
 - Add the standard or internal standard mixture of known concentration on wet ice and homogenise for an additional 2-3 cycles until the tissue is fully homogenised.
 - At this stage keep the homogenate on wet ice throughout the procedure.
- 12 Centrifuge at 4 °C at low speed (0.5 × g) for ~30 seconds to collect material from the tube walls.

Do not centrifuge at higher speeds or for longer durations to avoid phase separation.
Keep the homogenate on wet ice throughout these procedure.

Preparing the Mimetic Tissue Model Plug

- 13 Fill the cooled mould with tissue homogenate using a positive displacement pipette.
- 14 Insert the pipette tip into the mould and dispense the homogenate in a single continuous step, where possible, to avoid layering.
- 15 Keep the mould on dry ice until the homogenate is fully frozen.
- 16 Once frozen, extrude the mimetic tissue plug by gently pushing the plunger. Wrap the plug in aluminium foil / labelled small plastic bag and place it in a –80 °C freezer for at least 3 hours or overnight to ensure complete freezing.

Preparation of Mimetic Tissue Blocks

- 17 Embed the frozen mimetic tissue plug in hydrogel to form a block suitable for cryosectioning, as the plug size is typically small.
- 18 Prepare hydrogel blocks according to the relevant hydrogel preparation protocol.
- 19 Store frozen mimetic tissue blocks at –80 °C until sectioning.

Notes and Considerations

- 20
- The tissue matrix should match the biological samples as closely as possible.
 - Homogeneity of analyte distribution is critical for reliable quantification.
 - Storage duration and conditions may affect analyte stability.
 - Mimetic tissue models do not fully replicate in vivo tissue microenvironments but provide a robust and practical approach for MSI method optimisation and quality control.

Health and Safety

- 21
- Handle biological tissues according to institutional biosafety guidelines.
 - Use appropriate PPE when handling solvents and cryogenic materials.
 - Dispose of biological and chemical waste in accordance with local regulations.

Protocol references

[Revised Preparation of a Mimetic Tissue Model for Quantitative Imaging Mass Spectrometry](#)
[Full article: Quantification and Assessment of Detection Capability in Imaging Mass Spectrometry Using a Revised Mimetic Tissue Model](#)

Acknowledgements

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