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Organoids, organ-on-a-chip, separation science and mass spectrometry: An update

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ABSTRACT

Organoids are 3D models of organs, grown in the laboratory from stem cells. An organ model grown/placed in microfluidic devices is commonly termed an "organ-on-a-chip". Organoids and organ-on-a-chip devices are becoming important tools for studying physiology, disease modeling, drug discovery, personalized medicine, toxicology, and organ development/embryogenesis.

We review how mass spectrometry is used for studying organoids and organ-on-a-chip-derived material. We first focus on proteomics, metabolomics/lipidomics, and hormones, typically discussing liquid chromatography-mass spectrometry (LC-MS) approaches. We then review work on mass spectrometry imaging (MSI) of organoids and discuss organ-on-a-chip coupled with mass spectrometry. The review focuses on research developments from the past four years.

Mass spectrometric analysis of organoids and organ-on-a-chip has allowed novel insights on development and disease of e.g. brain, liver, and tumors, demonstrating potential for replacing or complementing animal models and other traditional model systems. Additional applications are emerging, e.g. related to sports doping and environmental toxicology.

1. Introduction

1.1. Organoids and organ-on-a-chip: lab-grown representations of human physiology

Organoids are laboratory-grown multicellular 3D organ models, which are emerging tools for studying physiology, disease modeling, drug discovery, personalized medicine, toxicology, and organ development/embryogenesis [1]. For organoids, cell sources such as adult stem cells or pluripotent stem cells (PSCs) are differentiated and organized into models of e.g. gut, brain, liver, lung, and kidney, depending on the biochemical/biophysical cues that are applied [2]. Also, tumor cells and tissue are being grown as organoids. Organoids can serve as alternatives to traditional cell cultures and animal models, which have limitations in representing human physiology. It is widely expected that organoids as more representative models of human disease will accelerate the speed and safety of drug development [3]. Organoids are also expected to play a powerful role in personalized medicine, as they may be grown from very specific sources, for example, cells derived from a single patient or cells harboring a mutation that represents a patient group. Organoids may also serve as therapeutic materials in near future: Corneal 3D tissue and insulin-producing islet organoids [4] are being developed for transplantation purposes.

An organ model that is grown and nourished on a microfluidic device is often called organ-on-a-chip (OoC) [5]. Prominent examples include the lung-on-a-chip, blood-brain barrier models, and liver-on-a-chip platforms. Efforts are ongoing to combine multiple organ representations by coupling several units to study inter-organ dynamics [6].

As organoids and OoCs have substantial possibilities for contributing to advancements in therapy they are rapidly becoming a focus in basic and applied research and the pharmaceutical

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industry. However, although new protocols are being developed at an incredible speed, organoids/OoCs are still in their infancy regarding functionality and complexity. In addition to a spectrum of analytical and imaging technologies that are used on organoids and OoCs, analytical chemistry tools provide valuable approaches to assess their functionality, complexity and response to interventions. In particular, mass spectrometry (MS) is a powerful tool for studying the content and chemical profiles of biological material, often combined with separation science such as liquid chromatography (LC).

1.2. Mass spectrometry: a powerful tool for measuring biomolecules

Mass spectrometry (MS), i.e. the gas-phase measurement of compounds' mass-to-charge (m/z) ratio, is a key approach for identifying and quantifying chemical compounds. Biomolecules (even large proteins) can enter the gas phase by using soft ionization techniques such as electrospray ionization (ESI) or atmospheric chemical ionization (APCI). In clinical and pharmaceutical settings, MS is routinely applied for targeted analysis, i.e., measurements of preselected compounds such as small molecule drugs and their metabolites. For an untargeted, comprehensive analysis, high-resolution (HRMS) instruments are required, such as time of flight (TOF)-MS and Orbitrap MS instruments. Today, HRMS allows for measuring up to thousands of biomolecules within a single run with various omics approaches such as metabolomics and proteomics. MS experiments often contain multiple MS-steps (MS/MS), i.e. MS analysis of intact compounds/fragmentation/MS analysis of fragments.

1.3. Separating compounds before MS can increase sensitivity and selectivity

Prior to MS analysis, compound mixtures (cell lysate, tissue extract, etc.) often have to undergo a separation step, so compounds enter the MS instrument at different time points, enhancing sensitivity and selectivity. The separation of biomolecules from organoids/OoCs (and other biosamples) is often performed using liquid chromatography (LC); using a solvent mobile phase, samples are pumped through a column or microfluidic channels, where compounds are separated based on different affinities to a stationary phase. Separation can be according to hydrophobicity (reversed-phase LC, the most common approach), ion strength (ion exchange LC), or other highly tunable variants such as hydrophilic interaction liquid chromatography (HILIC). Conventional LC columns have inner diameters (ID) between 2.1 and 4.6 mm. For the analysis of limited samples, the columns ID is downscaled to ca. 50–100 μm (nanoLC), and for extremely limited samples (for example, single cells) the ID may be as low as 5 μm [7]. Lower IDs require low flow rates (lower nL/min range) which also enhance electrospray ionization efficiency and hence sensitivity. On the other hand, the small ID format traditionally has reduced robustness, and compromises are being explored in clinical proteomics [8].

1.4. MS imaging: viewing the distribution of compounds in an intact sample

For analyzing the distribution of chemicals within an intact sample, MS imaging (MSI) is an option. With MSI, fixed samples (e.g. a liver or tumor slice) are scanned. Compounds from different regions of the sample section are sequentially ejected to MS and measured until the entire sample section is scanned. MSI is performed by e.g. electrospraying the sample point (desorption electrospray ionization = DESI) or by shooting the sample point with a laser beam (matrix assisted laser desorption ionization = MALDI).

1.5. Sample preparation

In addition to separation and mass spectrometric analysis, samples typically have to undergo preparation steps for clean-up and/or enrichment, both to ensure satisfactory measurements and to protect the analytical equipment. Similar to the steps required for other biosamples, organoid/OoC sample preparation steps typically target the analyte group of interest, e.g. by cleavage of proteins to peptides for "bottom-up" proteomics or extraction with hydrophobic solvents for lipidomics. Commonly, sample preparation and LC-MS are physically separated. However, recent advances allow combining organoid culture, sample preparation, and LC-MS in an integrated system, often referred to as being "on-line" [9]. On-line systems allow e.g. higher automation but often add complexity.

1.6. Review objective

The objective of this review is to discuss research from the past 4 years that employs MS-based approaches for studying organoids and OoCs (Fig. 1). Our focus area encompasses proteomics, lipidomics, and hormone/peptidomics. Much of the research discussed in this review is based on LC-MS, but we also include a section dedicated to MSI and a section discussing the coupling of MS and OoCs. In these last two sections, several applications of drug distribution and metabolism are also discussed. We highlight papers that we consider pushing boundaries technologically or methodologically, rather than focusing on purely routine applications of MS. For additional background, see our 2019 review [10], and Murphy and Sweedler's recent review on organoids and metabolomics [11].

2. Overview of recent research developments

Fig. 2 provides an overview of organoid and OoC research using MS, showing the number of categorized publications in the years 2011–2022. Recent research developments (2019 onwards) which are covered in this review are colored in orange. Although MS was already used for studying organoids and OoCs models before 2019 [10], the recent four years have seen a far wider range of organs/substructures to be researched. Intestinal models are the most studied with MS, followed by brain, liver, and pancreatic models. Common research interests are diseases including tumors. To a lesser extent, drug development is featured. Proteomics is the most commonly used omics approach, followed by metabolomics and lipidomics. Peptidomics and MSI are yet to be widely applied for organoid and OoC models.

2.1. Proteomics

Proteomics is typically associated with the global analysis of a sample, focusing on the detection, identification, and measurement of thousands of proteins. Approaches can also focus on particular post-translational modifications (e.g. phosphoproteomics) or predefined analytes (targeted proteomics). Proteomics has reached a considerable level of analytical validity and is emerging as a routine tool for clinical applications.

Proteomics is also an established tool for studying organoids [17], suited for e.g. benchmarking organoid protocols with human tissue/organ samples, studying effects of organ perturbations (e.g. modeling reperfusion injury through exposing intestinal organoids to hypoxia–reoxygenation or exposing brain organoids to illicit drugs), or tracking molecular dynamics in organ development. Deep (but time-taking) proteomics can today allow over ten thousand proteins to be analyzed in an organoid sample. However,

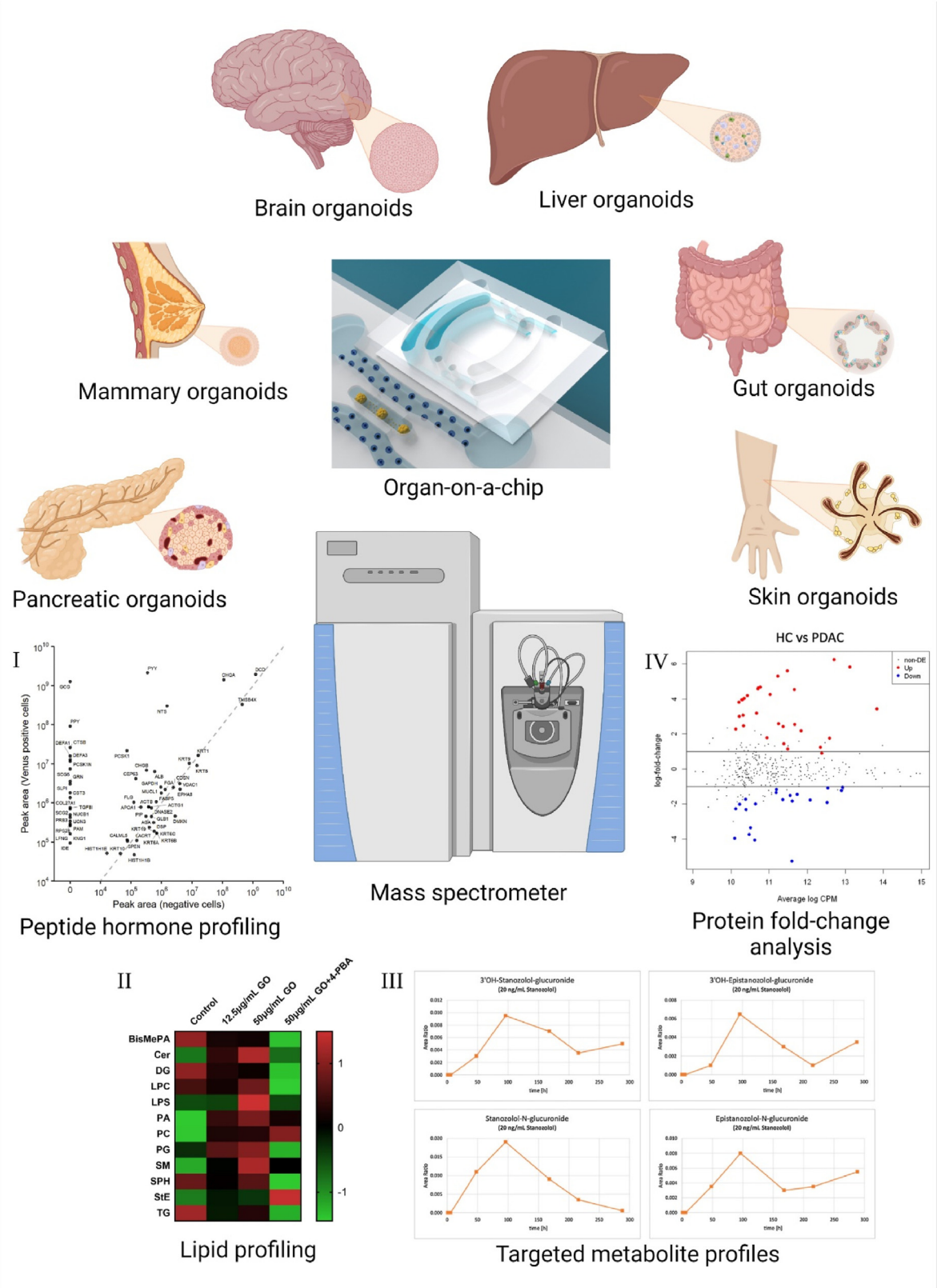

Fig. 1. Organoids and MS. Illustrative drawing of organoids grown in the laboratory to represent different types of tissue from e.g. pancreas, mammary glands, brain, liver, gut or skin. The organoids may also be studied on organ-on-a-chip models, here represented by a pump-less recirculating organ-on-a-chip platform [12]. By applying MS on the organoids, a wide range of information may be obtained concerning e.g peptides, proteins, metabolites, and lipids. For example, (I) by comparison of gut hormones present in ileal organoids with L-cells or without L-cells, MS-based peptidomics confirmed that the L-cells contained an enriched level of gut hormones [13]. (II) Lipid profiles of brain organoids exposed to graphene oxide nanomaterials were examined by untargeted lipidomics and it was shown that the contamination altered the lipid profile [14]. (III) Concentration profiles of stanozolol phase-II metabolites were demonstrated in liver organoids kept on a microfluidic chip [15]. (IV) Proteome profiles obtained from exosomes produced by cancerous and healthy pancreatic organoids revealed candidate proteins with significant change (Up or Down) or without differential expression) [16]. Adapted from Refs. [13,15,16] with permission under the Creative Commons Attribute licensing. Adapted from Ref. [14] with permission from Elsevier.

numbers are typically around 5–6 thousand for routine analyses, with each LC-MS run lasting a few hours (samples can, however, be run in parallel with various labeling approaches) [18]. The speed of proteomics experiments is increasing, and recent approaches allow thousands of proteins to be detected within minutes using data-independent acquisition approaches [19].

Proteomics is typically performed using nanoLC columns and is compatible with small amounts of samples with limited availability. Single organoid proteomics is possible and has been used to gain an understanding of the origin of SARS-CoV-2-induced neurological disorders [20].

Another example of proteomics on organoids is studying extracellular vesicles released from organoids. For example, exosomes produced by cancerous versus healthy pancreatic organoids were clearly distinguishable at a proteome level, potentially allowing for new avenues in disease progression studies, biomarker discovery, and diagnostics [16].

Proteomics can also be used to study the effects of environmental pollutants on organoids, thus creating an approach to predicting toxicity in humans. One example is the exposure of bisphenols to mammary organoids, which revealed tumorigenic effects [21].

Organoid-based proteomics is a highly versatile approach that is now used for studying a broad range of areas. Proteomics can be used also for very limited sample sizes related to organoids, and deeper understandings may be established with less-routine approaches such as ultra-narrow LC-MS to study single-cell studies of organoids.

2.2. Metabolomics/lipidomics

The combination of organoids and metabolomics (and its subcategory lipidomics) is an emerging approach for e.g. disease modeling and drug development. An overview of recent research on MS-based metabolomics/lipidomics of organoids is given below, with examples from the brain, tumors, and skin. In an example related to Alzheimer's disease, Glasauer et al. performed targeted lipidomics analysis of cerebral organoids using multiple reaction monitoring (MRM) and APCI-MS. They showed elevated levels of cholesterol and its precursors in mutant cerebral organoids, implying a correlation between dysregulated cholesterol metabolism and neurodegeneration [22].

Using untargeted approaches, Neef and Janssen et al. optimized the method for metabolic and lipidomic profiling of extracellular matrix (ECM)-based organoid cultures (<500 cells/injection). The optimized method was then used for phenotyping metabolic drug response in colorectal cancer organoids [23]. Regarding sample preparation, a key optimization was achieved by minimizing interferences from the extracellular matrix.

Another study used untargeted LC-MS lipidomics to demonstrate that the lipid signature in mouse sebaceous gland (SG) organoids resembles that in SGs *in vivo*, which implies that the organoids could provide a useful platform to identify potential therapeutic targets [24]. Untargeted lipid profiling by LC-MS has also been used to study the effects of adenomatous polyposis coli mutations on lipid metabolism in intestinal organoids [25].

Steroid structures have also been studied using organ models. Görgens et al. used liver spheroids (organoid-like structures created of mature adult cells) to test whether they can imitate human physiology for studying the metabolism of stanozolol and dehydrochloromethyltestosterone for sports drug testing [15]. Collecting samples off-line, they found that metabolite patterns matched commonly found urinary patterns of metabolites upon incubation of up to 14 days on a chip.

Liu et al. determined the lipid profile of human brain organoids to examine the consequences of exposure to graphene oxide nanomaterials [14]. By untargeted lipidomics, their study showed that contamination altered the lipid profile, and hence disrupted the lipid homeostasis. This is an example of how organoids are becoming tools for studying the potential toxicological effects of nanomaterials and pollutants.

In disease modeling, mass spectrometric metabolomics/lipidomics can be combined with a plethora of techniques (transcriptomic, metabolomic, and pharmacological analysis). In a recent example, Kimura et al. performed a highly comprehensive study of metabolic-associated genetic susceptibility to non-alcoholic steatohepatitis [26]. Using an Xbridge Amide column for HILIC separations, the authors observed an increase in lipogenesis and lipid oxidation markers in the liver organoids with induced steatohepatitis. As another example, using patient-derived colorectal cancer organoids as a disease model, Sugimura et al. demonstrated an antitumorigenic effect of the probiotic bacterium *Lactobacillus gallinarum* [27]. Using LC-MS for identifying active compounds, the authors attributed the tumor-suppressing effect of *L. gallinarum* to its production of indole-3-lactic acid. LC-MS may also be used to confirm the validity of other measurement approaches. An example is by Zanetti et al., who used targeted LC-MS to selectively detect dopamine and other neurotransmitters to verify results obtained with an electrochemical sensor [28].

Metabolomics/lipidomics and organoids are today frequently matched, using a rich variety of organ models and LC-MS variants (various column types, targeted/untargeted analysis, etc.). Typically, conventional column sizes are employed. Additional gains in sensitivity with small samples may be possible using narrower bore columns, such as nano LC used in proteomics.

2.3. Hormones/peptides

Hormones span a broad range of biomolecular structures, and analysis must often take into account particular traits of the targeted analytes (e.g. the “stickiness” of hormones such as insulin). Organoids have been shown to be a suitable vessel to enable studies on specific hormone-producing cells that are found in very low abundance in human organ tissue. For example, a wide range of gut peptides are produced in enteroendocrine cells (EECs); however, EECs comprise of approximately 1% of the intestinal epithelium, making it difficult to examine changes in specific hormone-secreting cell types. Nevertheless, human ileal organoids allowed long-term maintenance of GLP-1-secreting L-cells, and MS-based peptidomics revealed that these organoids produced and secreted a range of gut peptides including the GLP-1 hormone [13]. In a study by Beumer et al. [29], LC-MS peptidomics was used for profiling endogenous prohormones and bioactive peptides (i.e. bioactive peptides are derived from prohormones through proteolytic processing) found in the intercellular peptidome of EEC enriched human gut organoids. The profiles were compared to mutant organoids, where various EEC proteases were knocked out by CRISPR-Cas9-mediated gene editing, and the study was successful in providing a model for determining the role of gut proteases in hormone processing and a disease model for defects in these processes.

A low abundance of hormones combined with limited organoid samples can present challenges in LC-MS method development. Olsen et al. first developed an on-line nano LC-MS platform for monitoring of insulin secretion from pancreatic islets to aid in the evaluation stages of stem cell-derived islet organoids and islets-on-a-chip development [30]. The attained detection limit of insulin (1 ng/μL) was insufficient for the determination of insulin secretion from the limited amounts of stem cell-derived islet organoids. The authors established that poor sensitivity was due to significant

Fig. 2. A summary illustration of organoid and OoC research using MS. Research articles (361 in total) on organoids/OoCs that referred to MS were retrieved from literature databases. The articles were computationally classified based on organs/substructures that are modeled as organoids/OoCs (y-axis), and occurrences of key research topics such as proteomics, and lipidomics (x-axis). The sizes of the spheres correspond to the number of publications. The colors represent publication trends, whereby research subareas with high recent publication counts are shown in orange. Specifically, the trend was calculated as the number of publications in recent years (2019 onwards) as compared to the years before 2019 and is shown in a common logarithmic scale with the upper and the lower limits of 1 and -1, respectively. This means that research subareas with >10 times more (or less) publication counts are colored in the brightest orange (or the darkest blue).

carry-over/non-specific adsorption to hardware. After replacing tubings and connectors, insulin secretion from the islet organoids was successfully studied, also using conventional LC (2.1 mm ID) [31]. Such adjustments may also be relevant for other analytes and analyte groups as well in organoid research.

2.4. Steps toward OoC MS designs

While organoids are today quite extensively studied with various MS approaches, OoC and MS is less frequently combined (Fig. 2). However, several interesting possibilities are being explored. Research on MS measurements of OoC models includes sampling from gut-on-a-chip, PSC-derived hepatic organoids, and iPSC-derived multi-organoids-on-a-chip (heart and liver) to assess the metabolic profiles of different models [32–34]. While the above examples are based on off-line sample collection and LC-MS measurements, on-line approaches (e.g. connecting the microfluidic systems of an OoC directly to MS) promise measuring minute analyte secretions in an automated fashion.

Bridging an OoC system fluidically with MS is not a straightforward process, and challenges can include decreased flexibility, poor robustness due to clogging, and incompatibility of the culture medium (high levels of salts, proteins, and ECM) with the downstream analysis [10]. Also, integrating the low-pressure *in vitro* devices with high-pressure LC separations has proven to be technically challenging, and early work on coupling *in vitro* microfluidic devices to MS relied on integrating simple solid-phase extraction (SPE) desalting before ESI-MS analysis [35,36]. Separating the two pressurized systems can, however, be made possible by introducing a modulating unit. Han et al. [37] combined a microfluidic chip with automatic MS analysis using an autosampler needle, where the sampling occurred from the well-shaped chip outlet. As a proof-of-concept, they studied the 7-hydroxycoumarin metabolism in monolayer cancer cells cultured in a microfluidic chip for 25 h, using sample filtering and conventional LC separations downstream to triple quadrupole-MS. Santbergen et al. studied the verapamil permeability of an intestinal barrier model, bridging the *in vitro* system directly to LC-MS analysis using three separate switching valves [38]. The first and second valves were used to collect the apical- and basolateral effluent from the intestinal barrier model, respectively, and the third valve modulated the sample transfer to the pressurized LC separations before QTOF-MS analysis. The same valve setup was later used in a study recreating parts of the gastrointestinal system [39]. To cope with the incompatibility of the salt content of the intestinal cell models, the authors used chip-based SPE nanotraps before separation and detection. Although analyte detection was possible using this setup, high MS background caused by the sample matrix was observed.

Total removal of culture medium seems to be challenging when using on-line SPE as the only sample-handling step. Thus, on-line sample preparation methods, which facilitate increased analyte selectivity, could be beneficially used for removing the MS-incompatible culture medium and the ECM. As an example of such preparation methods, Skottvoll et al. examined electromembrane extraction (EME) as a selective sampling technique for coupling liver organoids and LC-MS in an on-line system [40] (Fig. 3).

Fundamentally, EME comprises electrophoresis of selected small molecules from a culture chamber (donor solution) across an oil-immobilized membrane (supported liquid membrane), to an MS-compatible solution (acceptor solution). The nonpolar supported liquid membrane makes EME highly suited for small hydrophobic bases. Therefore, methadone was used as a proof-of-concept candidate for on-line MS analysis to study the kinetics of drug metabolism of liver organoids [41]. Liver organoid-methadone incubation and EME sampling were performed on-chip, while the LC sampling and separation were bridged using a single switching valve. With this setup, the authors were able to monitor the conversion of methadone by liver organoids to methadone metabolites for 24 h using QQQ-MS and miniaturized LC separation (0.5 mm column ID).

For on-line coupling, additional formats are under development. Kogler et al. [42] have developed an “organ-in-a-column” where hepatic organoids were loaded into LC housings (0.5 mm inner diameter). This device was made solely of commercially available LC parts and was coupled with an upstream pump, delivering medium and drugs, and a downstream valve system controlling sample collection and injection for LC-MS analysis. In the proof-of-concept study, the authors measured on-line the metabolism of heroin to 6-acetylmorphine and morphine in hepatic organoids.

2.5. MS imaging

To fully understand the morphology and physiological states of organoids, the spatiotemporal distributions of chemical compounds may need to be analyzed using MSI. MALDI seems to be the preferred ionization technique for MSI of organoids, as its high spatial resolution on commercially available instruments is beneficial for studying small organoids. Pioneering work with MSI of organoids using MALDI-MS has been undertaken by the Hummon group, summarized in a recent review [43].

New developments in MSI technology have enabled the imaging of small samples such as organoids (often ~500 μm). Low μm MSI spatial resolution can be achieved today with commercially available instrumentation. Resolution, and paths to improving resolution, are dependent on e.g. laser type and wavelength (MALDI) [44], capillary diameter and liquid bridge stability (DESI/nanoDESI) [45], and the minimum rastor of the sample stage. However, improved resolution can come at the cost of decreased sensitivity and longer sample acquiring/scanning time. Additional challenges in combining MSI and organoids include background noise from the basement membrane extract (BME, an essential ECM) on which organoids are grown. The BME for growing organoids is often Matrigel, which contains ECM proteins and growth factors that can give batch-dependent levels of background noise, leading to reduced signals from small molecules and peptides. Johnsen et al. addressed these challenges by centrifugation of tumor-derived organoids from Matrigel into a gelatin microwell microarray format before MSI [46]. In this way, they also avoided the challenge associated with multiple organoids being present at different depths (z-axis) in the Matrigel. Bakker et al. made a protocol for dissociating organoid clusters and removal of ECM [47], including strategies to obtain *m/z* distribution images, morphological images, and MS/MS data.

With MSI, drug penetration into organoids can be monitored helping to understand distribution and bioavailability. Recently, Liu et al. [48] used MALDI-MSI to examine the spatial distribution of the prodrug Irinotecan (used for the treatment of colorectal cancer) and its metabolites in patient-derived colorectal tumor organoids with a spatial resolution of 35 μm. They observed a time-dependent penetration of Irinotecan and that the active metabolite SN-38 did not co-localize with either the parent drug or inactive metabolite SN-38G, showing cell-specific metabolism of the drug (Fig. 4). Another protocol for studying drug penetration in colorectal cancer spheroids by MALDI-MSI has been described by Machálková et al. [49]. Distribution of perifosine, an inhibitor of the Akt pathway was detected with MALDI-MSI and overlaid with images from fluorescence immunohistochemistry (IHC) to localize cell-specific markers of viability, proliferation, apoptosis, and metastasis using fiducial points. A similar approach was also used for mapping antibodies

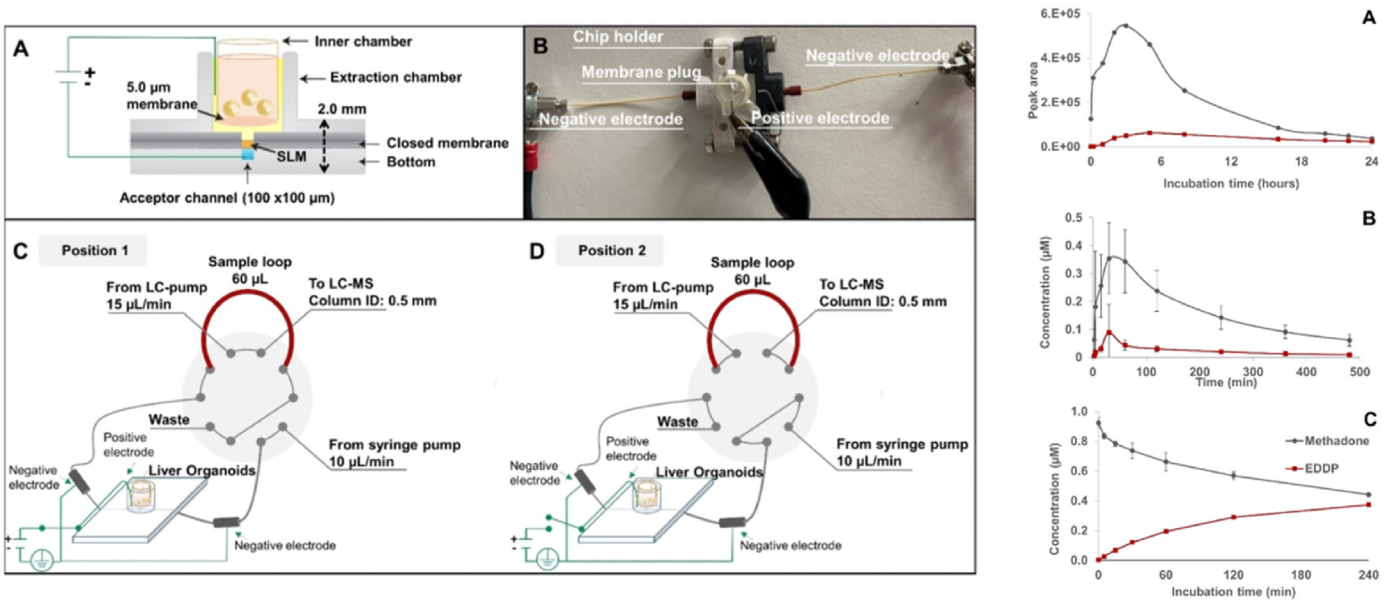

Fig. 3. Direct electromembrane extraction-based MS. Left: A) Figure describing liver organoid-containing chip, featuring electromembrane extraction (EME) for selective extraction of drugs and metabolites. B) Photograph of the prototype chip. C) Figure describing the connection to LC-MS. Right: Time plots of conversion of methadone to EDDP metabolite with direct EME chip (A), rats (B), and microsome (C). Reprinted from Ref. [41] with permission under the Creative Commons Attribute licensing.

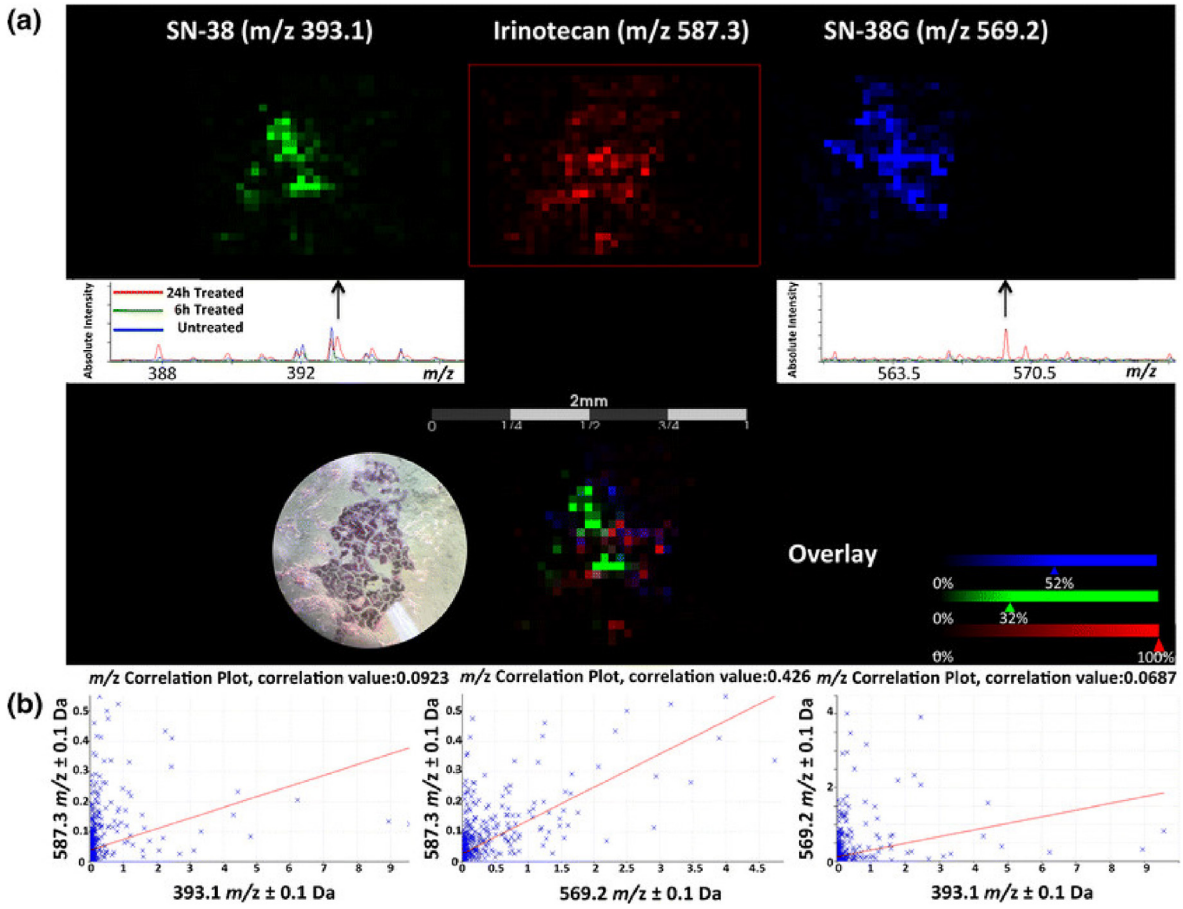

Fig. 4. A) MALDI-MSI ion images of the localization of the prodrug irinotecan and its metabolite SN-38 (active) and SN-38G (inactive) in tumor organoids. B) Correlation analysis of the localization. The active and inactive metabolites do not co-localize with the drug. Reprinted with permission from Ref. [48].

used in immunotherapy for colon cancer [50]. Antibodies were *in situ* reduced and alkylated to break disulfide bonds and to generate smaller fragments to detect the large therapeutic antibodies. Drug penetration has also been monitored using MALDI-MSI in blood-brain barrier (BBB) organoids, with a protocol for preparing the BBB organoids published by Bergmann et al. [51].

DESI has the advantage of being an ambient ionization source, and no vacuum is needed. However, the spatial resolution on commercially available DESI instruments (40–200 μm) is mostly insufficient for MSI of organoids. Even so, Flint et al. [52] combined DESI-MS, Imaging mass cytometry, and laser ablation inductively coupled plasma-MS on 3D cell culture for the in-depth understanding of tumor microenvironment, providing information on metabolite-, protein- and metal content. The DESI ionization source can be modified or replaced with a nanoDESI, where spatial resolution in 10–100 μm has been reported, making it a promising technique for the analysis of small molecules (<2000 m/z) from organoids.

The data generated with MSI experiments call for advanced analysis methods. An option for processing the data automatically is machine learning. Tian et al. [53] demonstrated that machine learning can be used to efficiently extract metabolic information from MSI of spheroids from colorectal cancer cells treated with Irinotecan. Hua et al. [54] utilized supervised machine learning to separate drug-treated and untreated spheroids.

4. Conclusions

In contrast to familiar biological samples such as urine and blood, the terms organoids and OoC include a diverse set of biomaterials. Due to the plethora of organoid types and OoC platforms combined, with often small sample sizes and volume, versatile analytical tools that can be tailored for the individual variant (or combination) of organoids or OoC are required. MS is such a highly versatile analytical approach, currently being used for a large range of studies of organoids and OoC, with a range from fundamental studies to preclinical applications. In particular, studies on drug metabolism and omics approaches promise to deliver data sets that have predictive power for treatment regimes in patients. MS and separation devices require significant operator skills and physical space, which may deter organ models from being coupled to on-line systems and create sample variation. On the other hand, MS systems are consistently becoming available in reduced sizes and complexity. Developments for merging *in situ* separation channels with organoid-containing culture devices or OoC devices will facilitate a broader use of MS technology in the field.

Although organoid research includes a wide variety of MS approaches, there is significant room for method development. This includes higher degrees of on-line monitoring and analyzing small amounts of samples with limited availability, such as single-cell studies of organoids. Separation steps prior to MS are crucial to sensitivity and selectivity. Liquid chromatography is widely applied in this context. Altered column sizes (nanoLC versus conventional UHPLC) and separation principles, e.g. reverse phase and HILIC variants can be explored and fine-tuned for increasing sensitivity, reducing sample sizes and for detecting specific metabolites and proteins. Additional approaches in separation science/MS would also be interesting to be investigated, e.g. top-down proteomics, capillary electrophoresis, and “on-chip” prepared monoliths. Considering the plethora of organoids and OoC platforms, it is obvious that one technology will not fit all purposes; Technological breakthroughs, such as developing robust nano LC systems for single-cell analyses of organoids, are highly welcome. Taken together, in the last years, the combination of organoids/OoCs, and mass spectrometry has drawn significant interest that is both driven by leaps in developing organ representations and technological advances in the MS arena.

Sample CRediT author statement

SRW, HRL: Conceptualization. JS: Formal analysis and Data Curation. SKo, KSK, CO, FSS, SRW, HRL: Writing - Original Draft. SKr, JS: Writing - Review & Editing. JS: Visualization. SRW, SKr, HRL, FSS: Supervision. Funding acquisition: HRL, SRW, SKr. The order of the first three authors (equal contributions) was decided by randomization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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