Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-022-04138-9

FEATURE ARTICLE

Solid-phase microextraction: a fit-for-purpose technique in biomedical analysis

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Received: 9 March 2022 / Revised: 2 May 2022 / Accepted: 17 May 2022

Abstract
Solid-phase microextraction (SPME) possesses unique features that allow it to be used in analyses that would not be possible with traditional sample-preparation methods. The simplicity of SPME protocols and extraction devices makes it a uniform platform for analyzing biological samples, either via the headspace or in direct immersion mode. Furthermore, flexible probe design enables SPME to be applied to target objects of different sizes, offering analysis on a scale ranging "from single cell to living organs". SPME microfibers are minimally invasive, which enables them to be applied for the spatial and temporal monitoring of target analytes or to assess changes in the entire metabolome or lipidome. Furthermore, SPME permits the capture of the elusive portion of the metabolome, thus complementing exhaustive methods that are biased towards highly abundant and stable species. Significantly, SPME can be interfaced with analytical instrumentation to create a rapid diagnostic tool. However, despite these advantages, SPME has some limitations that must be well-understood and addressed. This paper presents examples of up-to-date applications of SPME, challenges related to particular studies, and future perspectives regarding the application of SPME in biomedical analysis.

Keywords Solid-phase microextraction SPME · Bioanalysis · Biomarkers · Metabolomics · Unstable metabolites · Direct coupling

Introduction

The wide variety of techniques and sample-preparation methods currently available in bioanalysis would seem to leave little room for new strategies, particularly since "standard protocols" have been well-validated and shown to provide reliable and satisfactory results. Therefore, the question becomes why or when we should employ a newer tool such as solid-phase microextraction (SPME), which is simple to use in practice, but requires a sound understanding of its fundamentals to obtain reproducible and trustworthy data. A number of publications have already summarized either the different aspects and features of SPME in general or its applicability to bioanalysis in particular [1–3]. SPME is well-known for its effectiveness in analyzing volatiles in different areas of analytical chemistry (e.g., environmental pollutants, food, flavour and fragrances); as such, it is natural that the first biomedical applications of this technology would focus on the analysis of volatiles via the headspace (HS). However, the development of coatings that are compatible with complex biological matrices has also led to the application of SPME for the extraction of non-volatile compounds via direct immersion (DI) into the studied sample. Although early bioapplications of SPME mainly focused on determining concentrations of drugs in biofluids, this focus has slowly shifted towards the analysis of endogenous compounds. For in vitro/ex vivo studies, researchers tend to perform either manual extractions with fibers, which are the most popular SPME geometry, or automated or semi-automated high-throughput analysis using thin films arranged in the form of 96-blade brushes. While the ex vivo analysis of biofluids is a convenient alternative to more traditional sample-preparation methods, SPME offers features that make it unique and enable options that were previously unavailable or very limited, for example, in vivo analysis. Constant advances in the development of new coatings, devices, and strategies for coupling SPME with analytical

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Published online: 24 May 2022

instrumentation have made this technology highly flexible and applicable in subsequent steps of preclinical and clinical investigations of matrices ranging from cell lines to intact living organs.

From cells to clinical samples—uniform extraction tool for extrapolation and translational study

SPME's flexibility frequently enables its use to perform extractions from living cells and clinical specimens to identify correlations in the volatilome and potential biomarkers of various diseases. One example of such an application can be seen in a series of studies wherein the SPME was used to search for potential biomarkers of renal cell carcinoma (RCC) [4–6]. At first, SPME was used to study the volatilomes in urine samples from patients with different subtypes of RCC, which were then compared to those of a healthy control group. From the initial 21 volatile organic compounds (VOCs) differentiating the two groups, the authors eventually selected two metabolites, which passed the further stages of validation [4]. The subsequent investigation of potential biomarkers followed two directions: (1) more in-depth characterization of the urinary volatilome of clear cell renal cell carcinoma (ccRCC), which is one of the most common subtypes of RCC [5]; and (2) the differentiation of five different RCC cell lines based on their VOCs and volatile carbonyl compounds (VCCs), followed by the identification of metabolites that can potentially be used to discriminate between RCCs with different histological subtypes and metastatic potential [6].

While HS-SPME is well-established and has become a standard approach in volatilome analysis, direct-immersion SPME (DI-SPME) is still only used irregularly, despite findings showing its ability to fill the gaps associated with "traditional" approaches. One of SPME's main advantages is that it allows the same biological system to be sampled multiple times thanks to its low invasiveness and negligible depletion (under certain conditions) [7]. In the recent work, time-course analysis of the cellular secretome using two- and three-dimensional cell models (2D — cells grow on flat surfaces as monolayers, and 3D — e.g., hanging drops, spheroids, or more complex systems mimicking in vivo conditions), as well as an in vivo model of mouse melanoma, was performed using biocompatible probes with 2 mm long hydrophilic-lipophilic balanced (HLB) coatings [8]. Although very preliminary, the findings of this work showed that the proposed method has good potential as a uniform sampling tool for extrapolation studies, and adheres closely to the 3Rs rule (replacement, reduction, and refinement) by reducing the number of animals required for a given experiment. However, the authors also highlighted discrepancies between the studied models; for example, they noted that a larger number of compounds were found in the in vitro 2D cell culture model compared to the other two models. This result could be due to the in vitro 3D model's (spheroids) relatively higher integrity compared to the 2D model, which may have prevented the release of inter-spheroid metabolites into the culturing media.

Capturing unstable species

With regard to the sampling of living systems (e.g., cultured cells, circulating blood, or organs), SPME offers a very unique feature: the integration of extraction and metabolism quenching. At the same time, SPME does not involve physical sample collection, which has led to its in vivo/in situ applications being referred to as "chemical biopsy." The phenomena of metabolism quenching is explained by the property of the coatings used on the probe, as their porosity permits only small molecules to penetrate the sorbent while simultaneously retaining large macromolecules (e.g., proteins). As a result, enzymes are unable to access the trapped small molecules, which means that compounds that would otherwise rapidly degrade in the collected biospecimen remain protected and can be detected. The possibility of extracting labile molecular species in vivo was first reported for the metabolic profiling of mouse blood [9]. Later, the phenomenon was confirmed during tissue sampling [10–12], which has opened a discussion about the reliability of post-mortem studies, especially in relation to organs known to be particularly susceptible to rapid biochemical changes induced by death (i.e., brain). In the most recent study in this area, Napylov et al. compared the oxylipin profiles in the brains of conscious, moving rats obtained via in vivo SPME with those obtained post-mortem with solid-phase extraction (SPE) [12]. The distribution of the studied lipids was dramatically different (Fig. 1), with a significant shift towards species known to be highly elevated during brain ischemia, namely, hydroxyeicosatetraenoic acids (HETEs). Some of the HETEs were only detected during post-mortem SPE, indicating that they form after brain death during the processing of the resected tissue. The authors were only able to identify 20 of the 52 oxylipins detected in vivo. Of the 32 unknown oxylipin species, only 14 were also detected in the post-mortem studies, while the remaining 18 were considered unstable and having undergone degradation during traditional sample treatment. On the other hand, the post-mortem data contained a large number of oxylipins that were not detected in vivo, thus suggesting their formation after the animals had been sacrificed.

In a different work, Lendor et al. conducted a detailed investigation of early death-induced changes in rat brains [10]. To this end, they used SPME to sample the hippocampi of rat brains both in vivo and post-mortem (immediately after sacrificing the animal and 30 min later). Their profiling included both the polar and non-polar metabolomes and lipidomes, with the results revealing alterations to 42 metabolic pathways

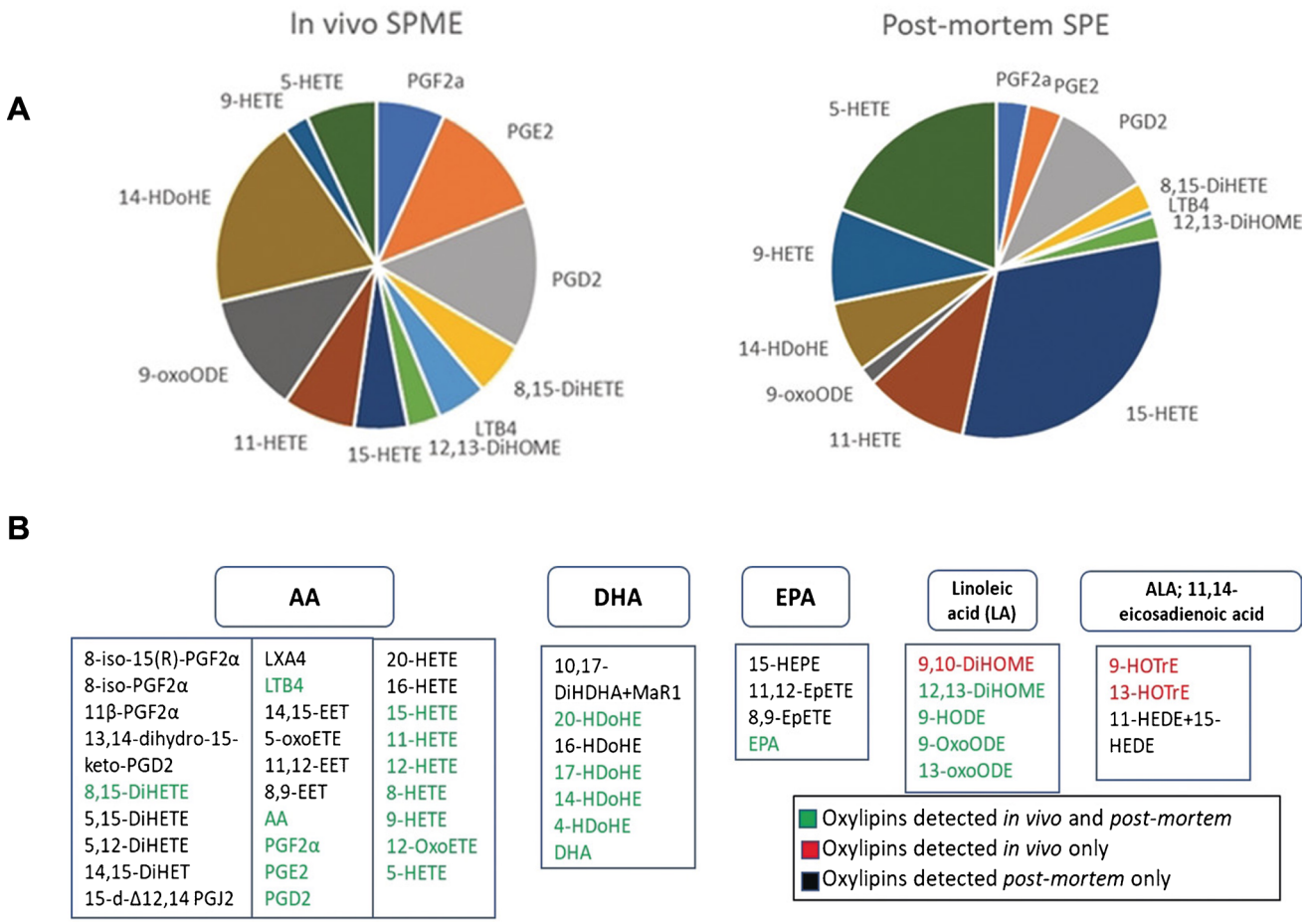

Fig. 1 **A** Comparison of the distribution (% by amount) of oxylipins detected by in vivo SPME and post-mortem SPE extraction of oxylipins in brain samples. The precursors, AA, EPA, and DHA, represent 98.3% of all species quantified via in vivo SPME and 99.6% of all species quantified via post-mortem SPE; thus, these species are omitted for clarity; **B** summary of all identified oxylipins observed using either in vivo SPME, post-mortem SPE, or both [reprinted from [12] with permission]

following death, over half of which being represented by more than one metabolite change. The most affected pathways identified based on the polar metabolome were energy-related processes, purine metabolism, and neurotransmitter generation. A variety of lipids including, but not limited to, glucosylceramides/galactosylceramides, phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, phosphatidic acids, lactosylceramides, monogalactosyldiacylglycerols, and prostaglandins also showed significant alterations that contributed to changes in neurotransmission/cell signal transduction, glycerophospholipid metabolism, and arachidonic acid metabolism among others. The findings of this work provided a number of key takeaways. First, the authors proved that the most significant changes in brain neurochemistry occur within the first 30 min post-mortem, which obviously influences the findings of post-mortem experiments. Moreover, the authors found that many of the observed processes, such as oxidative stress or pronounced inflammation, have already been reported as mechanisms that underly or accompany various diseases (e.g., Alzheimer's or Parkinson's diseases). Therefore, the authors recommended that any post-mortem study aimed at discovering biomarkers should be preceded by the identification of metabolites and pathways that are altered by death. These results should then be used as a filter during the interpretation of the disease-related factors.

Yu et al. utilized the GlobalStd algorithm and structure/reaction directed analysis in a linear mixed model to identify differences between sampling methods (in vivo SPME vs. ex vivo SPME vs. solid–liquid extraction (SLE)) and storage conditions (storage on fiber vs. storage of SPME and SLE extracts under different temperatures and storage times) [11]. In addition to examining changes at the molecular level, the authors also assessed the influence of the above-mentioned factors at the reaction level. The study was performed using fish muscle and the analysis was focused on the detection of unstable species. Yu et al.'s findings revealed that ex vivo and in vivo SPME captured similar metabolic profiles, and that distinct differences were observable in 13 and 14 metabolites

only when on-fiber storage and extract storage (desorption solvent with desorbed analytes) were used, respectively. When subsequently compared SLE to ex vivo, in vivo with on-fiber storage, and in vivo with extract storage, the numbers of analytes were 254, 223, and 253, respectively. While this result can be explained by the different fundamentals of the tested methods (i.e., exhaustive vs. non-exhaustive extraction), the authors did not specify which compounds contributed to the discrepancies. One important practical observation was that none of the tested analyte-storage strategies (i.e., on-fiber and in extract) had a noticeable effect on the metabolic profile. Rather, storage time (1, 3, 7, 14, 30 days) was found to have the most significant impact on the studied metabolome, while the impact of temperature (− 80 °C, − 20 °C, and 4 °C) was negligible. At the reaction level, the observed changes were largely related to lipids (e.g., the opening or forming of a double bond or the loss of two carbon atoms). Interestingly, the authors stated that it is possible to quantify unstable species based on reaction-level changes without identifying them. This is a very important development, as available databases lack vast information about transient metabolites. The ability to perform sampling in vivo and to monitor changes at the reaction level opens new opportunities to identify unrevealed portions of the metabolome and biochemical pathways.

Low invasiveness of SPME in clinical practice

SPME offers low invasiveness due to the small size of its probes (e.g., fibers), which has created the possibility of its application in clinical practice, mainly in the analysis of tissues/organs in vivo and in situ (Fig. 2) [3]. As previously discussed, traditional sample-preparation methods rely on the collection and homogenization of tissue, followed by the extraction of analytes with aqueous and organic solvents. While such approaches are widely used, their complex protocols make them unsuitable for on-site extraction (e.g., in the operating room, OR). Furthermore, tissue collection itself usually restricts an analysis to a single sampling because repeated biopsy carries the risk of tissue damage and other side effects. SPME was first tested in preclinical studies using a porcine model to monitor the function of different grafts in the peri-transplantation period [13]. Although the availability of organs is the major challenge associated with transplantation, the lack of effective preservation methods and reliable non-/low-invasive tools for assessing organ quality are also significant issues. In general, indirect methods are used to evaluate graft quality, while the histological assessment of a single biopsy is allowed in some cases for direct tissue analysis [14]. However, it is known that a graft undergoes significant biochemical changes (e.g., related to ischemia or oxidative stress) during its preservation, which can last for hours. Early investigations of SPME's effectiveness in monitoring rabbit kidneys during long-term cold preservation found significant differences between the changes in the metabolome during the first 4 h of cold storage versus those occurring up to 21 h later [15]. This finding aligned well with reports in the literature describing changes within the first few hours as a dynamic response to reactive oxygen species and so-called renal ischemic preconditioning. Later, SPME was shown to be an effective tool for the temporal monitoring of changes to the metabolome and lipidome in a given organ, as well as for discriminating between grafts subjected to different ischemia times (i.e., donors after cardiac death (DCD) vs. heart beating donors (HBD)) [13, 16]. The study indicated the potential of developing a panel of biomarkers comprised of both polar and lipid species for monitoring the status of the transplanted organ, as well as for identifying factors involved in ischemia/reperfusion injury and processes occurring during different preservation strategies (e.g., static vs. dynamic, hypo- vs. normothermic perfusion).

The first translational study from a porcine model to human subjects focused on the monitoring of the anti-cancer drug, doxorubicin (DOX), in lungs during a procedure known as in vivo lung perfusion (IVLP) [17]. During IVLP, a high dose of DOX is delivered locally to the lungs to target residual micrometastatic disease during tumor resection. One clear benefit of this strategy is that it avoids broader systemic side effects associated with this drug, as the DOX is not distributed outside of the lungs. Nonetheless, there are also challenges associated with IVLP, such as optimizing the dosing regimen to ensure that therapeutic concentrations of

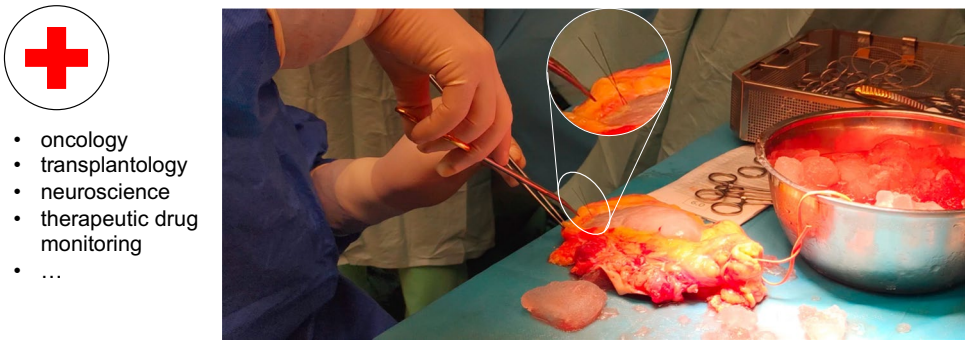

Fig. 2 The list of exemplary areas of medicine explored with in vivo SPME to date and sampling of kidney during transplantation procedure as an example of minimum invasive in vivo/in situ SPME extraction of human organ

the drug in the target organ are both safe and effective. In the preclinical studies, different DOX dosages and perfusion times were tested on pigs to determine the most suitable protocol. Typically, the concentration of DOX in the lung is usually determined by collecting a piece of the perfused lung tissue at the end of the experiment, followed by homogenization, solvent extraction, and HPLC analysis coupled with fluorescence detection. Conversely, SPME enables temporally and spatially resolved analysis, which allows clinicians to obtain information about changes in drug levels in different lobes of the lungs throughout the entire procedure. In addition to determining DOX concentrations in the lung, the authors analyzed changes in the levels of DOX metabolites as part of untargeted profiling aimed at monitoring the influence of DOX on lung tissue and selecting potential biomarkers of drug toxicity.

As discussed above, SPME has great potential as an innovative tool in neuroscience. The findings of early rodent studies [10, 12] enabled the application of SPME in in vivo brain studies in primates [18–20]. In their work investigating the interdependence between several neuromodulators and their effects on local circuits during goal-directed activity in macaque monkeys, Hassani et al. were able to use SPME to simultaneously extract four neurotransmitters: glutamate, dopamine, acetylcholine, and choline [18]. In a different experiment, Hassani et al. used a previously validated SPME strategy to measure donepezil and choline, a metabolite of acetylcholine (ACh), in the prefrontal cortex and anterior striatum of monkeys [19]. Their goal in doing so was to verify their hypothesis that donepezil enhances the cognitive domains of attention and flexible learning via area-specific ACh profiles. The results obtained in rhesus monkeys showed that, within a given dosage range, the drug causes a trade-off wherein attention declines and cognitive flexibility improves.

In vivo SPME was also applied in patients undergoing brain tumor biopsies [20]. In this work, sampling was performed with a device consisting of four fibers of two different lengths, which enabled the extraction of analytes from two locations simultaneously. In addition to characterizing the metabolomes and lipidomes of gray and white matter, this study sought to serve as proof-of-concept that SPME could be used to obtain the spatial resolution of specific brain areas in a relatively short time (4 min) and to provide a representative set of compounds that permit the differentiation of target structures. The findings of the above study enabled to envision that healthy tissue and cancerous lesions in the same individual can be sampled simultaneously for the purpose of brain tumor diagnosis, which has been supported by the results of a recent study wherein SPME was used as an on-site extraction tool for brain tumor characterization [21–23]. In the papers generated by this latter study, it was concluded that the proposed strategy (in situ extraction of resected tumors in the OR) enables the discrimination of brain tumors of different origins; the classification of a tumor based on its malignancy grade; and the identification of metabolic and lipidomic phenotypes reflecting genetic mutations, which are established biomarkers of gliomas [21–23].

Miniaturization of SPME devices

The above examples show that, although fiber-based SPME probes are small and cause minimal damage to the studied system, particularly in applications like in vivo brain studies, there remains a need for devices that can fit in the space between blood vessels, as such devices would ensure that the studied tissue metabolome is not contaminated by blood metabolites [10]. There is also a question of damage to individual cells caused by inserting the probe into the tissue; while this phenomenon is not currently being explored, one may speculate that it is similar to other techniques, like microdialysis. On the other hand, the findings of the aforementioned oxylipin studies showed no increase in arachidonic acid or proinflammatory prostaglandins, thus indicating that in vivo SPME sampling with regular (i.e., ca. 290 µm diameter) probes does not evoke an inflammatory response [12]. In an attempt to minimize tissue damage during the sampling of nonhuman primate brains, Lendor et al. modified SPME probes by recessing 3 mm of the tip of the stainless steel wire to obtain a diameter of 100 µm (150 µm before recession) and coating it with 1.3 µm HLB-SCX particles, which were selected for their superior properties in the extraction of the targeted neurotransmitters [24]. The developed probes were then used successfully in the previously mentioned applications conducted in monkeys [18, 19].

While reducing the invasiveness of sampling is a major driver of the miniaturization of SPME devices, targeting small objects, including single cells, is another. The main strategy for preparing miniaturized SPME probes is to etch the tip of the wire that serves as a metal support. As discussed by Piri-Moghadam et al., the tip provides a rapid rate of enrichment, which allows equilibrium to be achieved for the majority of the analytes, even under static extraction conditions, due to radial diffusion [25]. Moreover, solvents with high affinity towards the analyte of interest enable fast and complete desorption, thus enhancing the sensitivity of the assay. In the same paper, Piri-Moghadam et al. proposed the use of polypyrrole (PPy) coated stainless steel microtips, which were prepared using acupuncture needles with a diameter of 120 µm. After etching, the diameter of the tip measured ca. 5 µm and the coating length, which was immobilized electrochemically on the needle, was 150 µm and 500 µm for small objects and larger-volume sampling,

respectively. The small objects sampled with these microtips included 1–10 µl of blood and PBS, 5 µl of urine, and single cells of *Allium cepa* L. (red onion). The quantitative results for the analysis of fluids showed very good sensitivity and precision, while only qualitative single-point data was obtained for the single cells [25]. In a different work, Vasiljevic et al. proposed a similar concept for quantitating nine drugs of abuse in 1 µl of blood; however, they used coatings prepared with hydrophilic-lipophilic balanced particles and biocompatible polyacrylonitrile [26]. Their results showed that this approach provided very satisfactory figures of merits, including low limits of detection ranging between 0.5 and 2.5 ng/ml. Taking advantage of HLB's good analyte coverage, Vasiljevic et al. then used their microtips for the untargeted profiling of individual caviar eggs from several different fishes. The results of these assays showed clear separation among samples of different origin, and the chemometric analyses enabled the identification of discriminating metabolites. Taken together, these results indicated this approach's potential for future use in discovering biomarkers in small samples/single cells in other bioapplications. Deng et al. also used an SPME probe with a tip less than 5 µm in diameter to perform in vivo, in situ, and microscale lipidomics [27]. Their findings demonstrated this device's ability to extract lipid species within the MW 750–1000 Da range, which is similar to shotgun MS. In this work, in vivo sampling was performed on zebrafish, while in situ sampling was performed on *Daphnia magna*. In the first case, the obtained lipidome allowed the authors to distinguish between the ovum and adult stage; in the second one case, the authors targeted different locations of the animal (i.e., abdomen, back, head, and tail), with results showing similarities in the detected species of lipids, but significantly different content and concentrations of these lipids at the various sampled locations. As one might expect, the miniaturization of SPME devices poses challenges in terms of sensitivity. Therefore, in the great majority of cases, the microprobes are coupled directly with analytical instrumentation to eliminate the dilution related to chromatographic separation. Most often—for instance, in the works cited above—the instruments used for this purpose are mass spectrometers [25–27], although other options have also been explored. For instance, in their recent work, Chang et al. integrated polypyrrole modified carbon fibers with a working electrode for the extraction of dopamine from single living cells followed by electrochemical detection [28]. In this work, the authors demonstrated that their developed nanoprobe, which had a conical tip with a diameter of less than 100 nm and a coating thickness of ca. 10 nm, can be successfully applied to perform repeated sampling on the same cell PC12 cell, followed by the offline detection of dopamine in the three-electrode system (Fig. 3). Their optimized setup permitted the detection of dopamine at a level of 10 pmol/L and allowed them to observe changes in the cytosolic concentration of dopamine induced by potassium cations.

Rapid and sensitive determination of target compounds

As mentioned above, the direct coupling of SPME with a mass spectrometer or other instrumentation enables sensitive and fast (ca. 2–5 min) analysis. Numerous interfaces and strategies have been applied to different SPME geometries, and these configurations have been summarized in various recent reviews on the topic [1], [29]. Some of these approaches, like nanoESI or microfluidic open interface (MOI), were designed primarily for use with fibers,

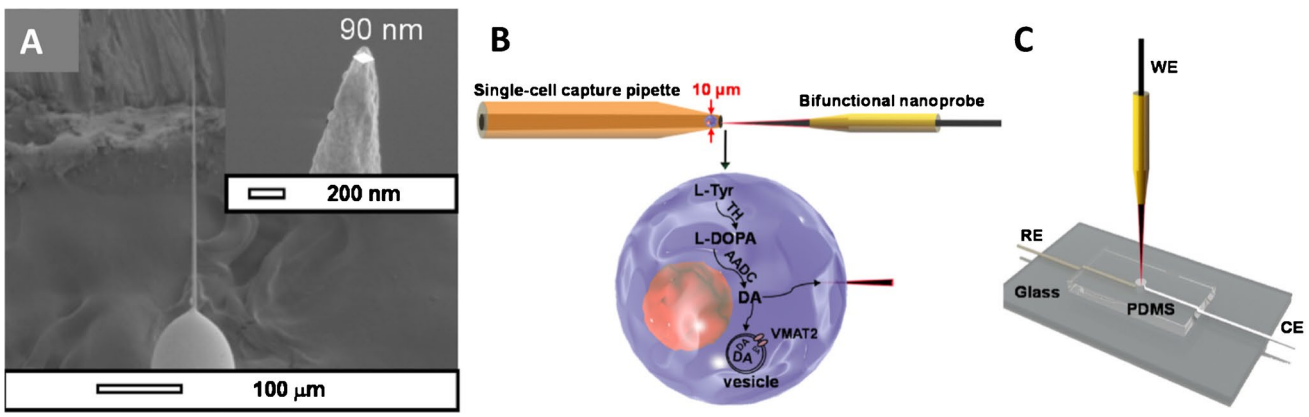

Fig. 3 Characterization of the bifunctional nanoprobe and its glass/carbon fiber interface and schematic representation of detecting cytoplasmic dopamine in a single living cell. **A** SEM image of the PPy-coated carbon fiber nanoprobe (the inset shows the tip of the PPy-coated carbon fiber nanoprobe); **B** the schematic of SPME of a living cell in a single-cell capture pipette by a bifunctional nanoprobe. **C** The schematic of electrochemical detection using an after extracted bifunctional nanoprobe as a WE. Reprinted from [28] with permission

while others like coated blade spray (CBS) are compatible with thin-film microextraction. Looby et al. validated their results attained with SPME-MOI-MS/MS against those obtained with SPME-LC–MS/MS, showing that the former approach offers a fast and effective method for determining tranexamic acid blood concentrations [30]. In contrast, Gomes-Rios et al. showed the potential of CBS-MS/MS for determining concentrations of immunosuppressants in whole blood samples, which is challenging because this group of drugs is characterized by very high (> 80%) binding to proteins located inside red blood cells [31]. The authors added an extra step (i.e., lysis of red blood cells, RBCs) to the routine SPME protocol, thus releasing an intracellular pool of the drugs and increasing the sensitivity of the assay. At this point, it should be emphasized that the main advantage of rapid SPME-based protocols—compared to other techniques that enable rapid interfacing with MS (e.g., paper spray, thermal desorption, or rapid fire)—is that they allow analytes to be introduced to the instrument quickly without compromising effective sample preparation. As a result, SPME provides very clean extracts for analysis instead of a dissolved mixture of all sample constituents (i.e., analytes of interest and interfering compounds). Nonetheless, the most common SPME-MS couplings are not the only options for directly interfacing SPME with analytical instrumentation. For instance, as discussed above, Chang et al. coupled SPME with electrochemical detection [28]. Furthermore, recent publications have detailed the coupling of SPME with a fluorimeter for the screening of doxorubicin [32]. This strategy was developed as an extension of work aimed at monitoring DOX in the lung during IVLP; as discussed above [17], this approach offers a cheaper and easier alternative to MS detection. In another example, the vinylpyrrolidone-based thin film microextraction coupled to direct solid-state spectrofluorimetry enabled fast, sensitive, simple, and high-throughput analysis of selected sartans in human plasma due to the characteristic properties of the developed coating [33]. On the other hand, Xia et al. used paper-based TFME for detection and quantitation of volatile benzaldehyde, a biomarker of lung cancer, in exhaled human breath [34]. For that purpose, the authors developed stimuli-responsive core–shell gold nanorod (GNR) quantum dot (QD)-embedded metal–organic framework (MOF) structures sorbent, which has a biomodal sensing capability, i.e., can be used for fast visualization of targeted analytes with the naked eye using fluorescence detection or accurate quantitation at the sub-ppb level with excellent specificity against other volatile organic compounds using surface-enhanced Raman spectroscopy (SERS) [34]. It is also worth mentioning work by Eitzmann et al. [35] who designed a thin film device for extraction of deoxyribonucleic acid (DNA) based on polymeric ionic liquid (PIL) coating immobilized on nitinol sheets. The proposed method can be faster and simpler alternative of isolating DNA prior to quantitative polymerase chain reaction (qPCR) as demonstrated by the authors during analysis of specific SARS-CoV-2 sequence spiked into artificial oral fluid samples. Moreover, the comparison of the results obtained for TFME and analogous SPME protocol showed superior performance of TFME in identification of positive, clinically relevant concentration, compering to SPME (100% and 67% of samples, respectively). The authors envision this TFME method to be coupled with microfluidic device or mass spectrometer for direct DNA analysis or to perform matrix-assisted laser desorption/ionization (MALDI) analysis directly from TFME device where PIL would act as a matrix [35].

The combination of simple extraction, minimal invasiveness, the possibility of in vivo and in situ sampling on site, and fast and low-solvent-consuming instrumental analysis make a compelling case for the inclusion of SPME-based strategies as rapid diagnostic tools in the OR or intensive care unit (ICU).

Conclusion

Solid-phase microextraction has a number of unique features that allow it to perform analyses that are not possible with traditional sampling, sample-preparation, and extraction methods. However, it is important to recognize that SPME is not meant to replace other approaches; rather, it is designed to serve as a complementary tool in the analytical portfolio. Detailed information about different aspects of SPME in bioanalysis can be found in the reviews on the topic [1–3, 36, 37]. As the examples discussed in this paper demonstrate, there is a distinct part of biology that remains unexplored. It appears that SPME is the tool that may help researchers tap into it. Considering low invasiveness and simplicity of probe operation as well as the opportunities for direct coupling to analytical instrumentation without chromatographic separation, it can be envisioned that SPME is a very good candidate to be used as a tool for rapid clinical analysis on-site. Among the limitations of SPME in bioanalysis, limited access to commercial devices for extraction of semi- and non-volatile analytes seems to be the main one. There are a number of publications reporting lab-made coatings of different kinds, which demonstrate the potential of bioSPME, but for users interested in ready-to-use devices, this remains a great challenge. Similarly, as mentioned in the article, one of the main factors behind the wide range of application of SPME is the flexibility of the device's geometries and shapes, which at this moment can be achieved primarily by in-house customization. Nonetheless, if SPME's full potential is to be realized, it will be essential to start thinking outside the box.

Declarations

Conflict of interest The author declares no competing interests.

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