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Research Note

Blotting paper as a disposable tool for sampling chemical residues from skin surface

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Collection of sufficient amounts of chemical residues and metabolites from skin in a non-invasive manner is challenging [1]. The available sampling methods are based on the use of various sampling techniques [2]: macroduct, solvent extraction, tape stripping, oil/membrane approach, implementing absorbing materials such as semipermeable skin patch, alcohol swab, cotton pad, textile, nitrocellulose membrane, or imprinting materials such as silica plate, polydimethylosiloxane film and derivatized silicon. In certain cases, specimen collection is invasive while some materials cause inconvenience due to the time-consuming sampling step. In the past few years, our laboratory developed hydrogel micropatch sampling method, which was successfully combined with mass spectrometry (MS) allowing for detection of skin metabolites [3], disease biomarkers [4], and topical drugs [5]. The previous method took advantage of an atmospheric pressure sample introduction to MS without pre-separation. In fact, such hassle-free interfaces are popular in the MS-related research work [6,7]. However, eliminating the chromatographic separation step complicates quantitative analysis of complex samples by increasing the risk of isobaric interferences. Another drawback of our previous method was fast drying of the hydrogel (agarose), what put an additional burden on the analyst (preparation of the probes a few hours before sampling skin). In an attempt to address these issues, we have been searching for a better sampling material, and we intended to use it in combination with a hyphenated analytical technique. In principle, one could consider hundreds of synthetic materials (*e.g.* polymers) as potential sorbents for skin sampling. While a major limitation of implementing new synthetic materials in clinical applications is their

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biocompatibility [8], various commercially available skin-care products can potentially be used to collect molecules present on the surface of skin for chemical analysis. In this study, we show that oil-control blotting paper can lift chemical species originating from a topical agent from skin in the quantities sufficient for subsequent analysis by gas chromatography (GC) coupled with MS (Fig. 1A). Due to the great popularity of plant-based insect repellents [9], we exemplify the use of the proposed protocol by investigating retention of such a repellent on the surface of skin. The knowledge of the retention kinetics of the related topical agents on the skin can be useful for the development of more effective formulations with long retention times, and—in consequence—for more efficient protection against infectious diseases spread by insect vectors.

To verify the applicability of blotting paper to skin sampling, we tested three commercially available blotting papers (linen fiber paper, charcoal linen fiber paper, polypropylene polymer paper). Printer paper (70 g m^{-2}) was used for comparison. The bright field and fluorescence micrographs of these materials reveal intrinsic microstructures (fibers or pores; Fig. 1B and S1). Such microstructures provide high surface-to-volume ratios, thus enhancing sorptive capacities of the bulk materials, and promoting absorption of the sampled fluids (*e.g.* moisture, sweat) by capillary action. The paper substrates were cut into 2 × 2 cm squares with scissors. The squares were subsequently washed in pure ethanol (GC-MS grade) to remove residual contaminants, and dried. Such paper probes were then used to collect analytes from the surface of porcine or human skin. Excised porcine skin fragments (~100 cm^2) were washed in tap water, deionized water, and placed on top of 0.5% freshly prepared agarose hydrogel loaded to Petri dish to avoid dessication (Fig. 1C, left). The porcine skin was then cleaned with medical wipe soaked with 75% ethanol. The Petri dish was placed in the incubator set to

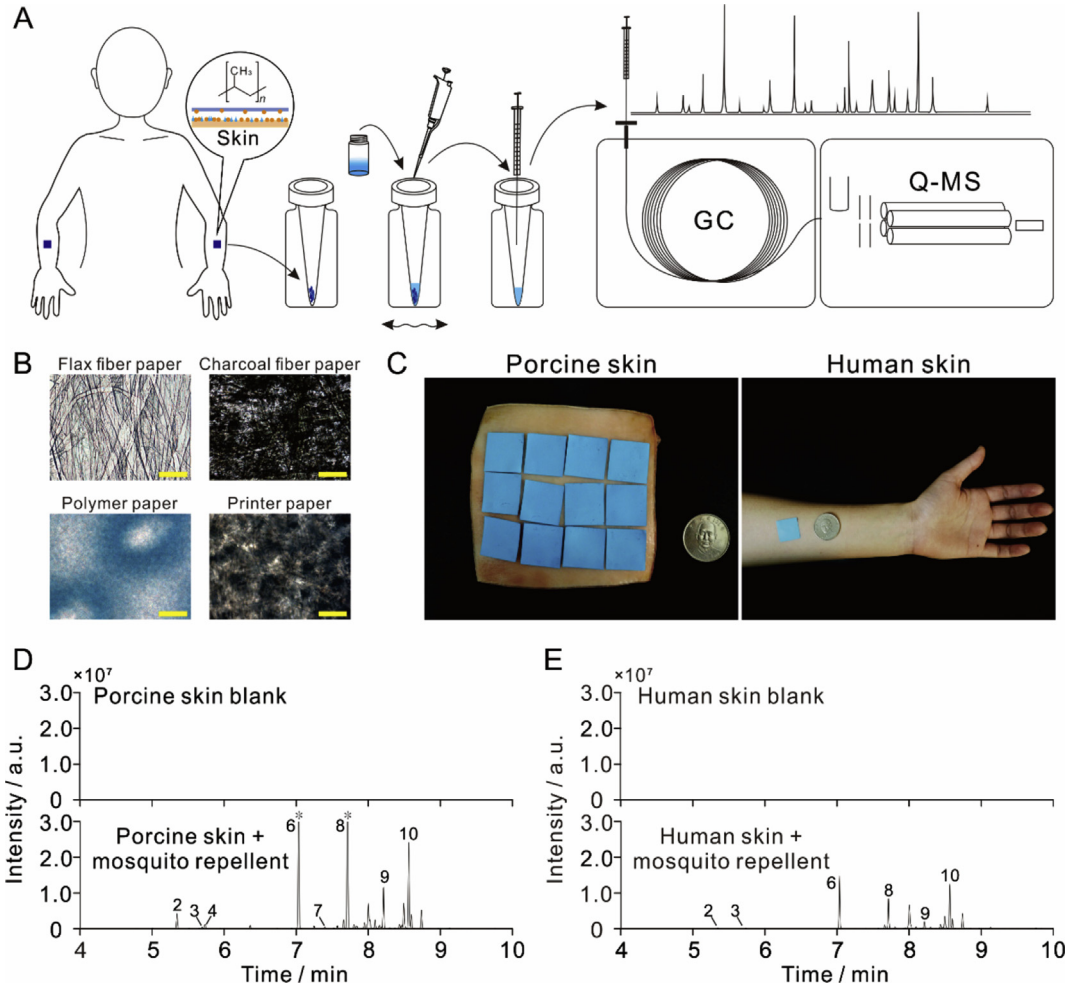

Fig. 1 – Blotting paper as a sampling probe for analysis of chemical residues present on the surface of skin. (A) Scheme of the sampling and analysis workflow. (B) Bright field images of four types of paper tested in this study. Scale bars: 100 μm. See Fig. S1 for larger bright field and fluorescence micrographs. (C) Specimen collection from excised porcine skin (*ex vivo*) and live human skin (*in vivo*). Note that several paper probes (here: 12) could be applied to the fragment of porcine skin at the same time to test different conditions of sample preparation. (D) Representative results obtained by sampling residues of mosquito repellent from excised porcine skin. (E) Representative results obtained by sampling residues of mosquito repellent from live human skin. Conditions in (D) and (E): exposure time, 10 min; sampling with polymer paper (2 min); extraction with acetonitrile (5 min); GC-MS EIC: *m/z* 93. Asterisks indicate the peaks that are higher than 3.0 × 10^7 a.u. The numbers correspond to the putatively identified compounds listed in Table S1 (very small peaks are not labeled).

37 °C for ~1 h. The porcine skin fragment was then sprayed with a commercial mosquito repellent (composition: eucalyptus extract, lavender extract, mint extract, lemon grass extract, citronella oil, water) from a distance of ~15 cm three times. Following a brief exposure (typically, 10 min), the sampling probes were applied to the porcine skin surface (typically, 2 min). The glass lid of Petri dish was placed on top of the probes to improve contact between the probes and the skin. Human skin (forearm) was sampled in a similar way following previous application of the same mosquito repellent (Fig. 1C, right). In this case, adhesive bandage was used to hold the paper probe in place when collecting specimen. The sampling was conducted at the room temperature (~20–25 °C).

The probes (paper squares) were then transferred by tweezer to glass vials with inserts (150 μL). Subsequently, 50-μL aliquots of a solvent (GC-grade hexane, analysis grade acetone, LC-MS grade acetonitrile, or HPLC grade isopropanol) were pipetted into the inserts. The vials were then placed in the mini-thermoshaker set to 30 °C and 1000 rpm (typically, 5 min). The extracts were transferred to new vial by micropipette and analyzed by GC-MS (Fig. 1A; see Supporting Information).

The obtained chromatograms in Fig. 1D and E reveal numerous peaks corresponding to the components of the mosquito repellent, which were lifted from the surface of skin by polymer (polypropylene) paper and re-extracted by acetonitrile prior to analysis. Based on the results shown in Figs. S2 and S3, this combination of paper type and solvent provides chromatograms with a large number of intense peaks (comparison for the same *m/z*; note that MS signals are also recorded at other *m/z*). Although—in some cases—acetone extraction (Fig. S3B) led to more intense chromatographic peaks than acetonitrile extraction (Fig. S3C), the high volatility of acetone makes it a poor extraction solvent. Following further optimization—and in an attempt to shorten the procedure—the sampling time was set to 2 min (Fig. S4), while the extraction time was set to 5 min (Fig. S5). Ten chromatographic features were putatively assigned to empirical formulas and compound names by comparing the electron ionization spectra and retention times with the corresponding spectra and retention times of chemical standards (Table S1). The dynamic ranges of the GC-MS method were verified by constructing calibration plots for these compounds (Fig. S6 and Table S2).

We further tested the influence of exposure time on the relative abundances of the sampled residues of the mosquito repellent on the surfaces of excised porcine skin (Fig. S7) and live human skin (Fig. S8). Interestingly, the obtained data series show temporal decays. To estimate the decay rates, we subsequently fitted these data series with an exponential function:

$$A = A_0 e^{kt} \quad (1)$$

The fitting parameters (A_0 and k) are listed in Table S3. One should note that the values of A_0 depend on various experimental parameters, including concentrations of the mosquito repellent component as well as ionization efficiencies. However, the decay rate constants k (with negative values) should mainly depend on the retention of these components on the skin surface. Thus, the signal decay is due to the processes such as chemical decomposition, absorption by skin, and evaporation from skin. Apparently, the k values (Table S3) are slightly correlated with the volatilities of the analyzed compounds approximated by the boiling points and vapor pressures (Table S1, Fig. S9). Their decay curves must be influenced by evaporation from the skin. However, one can also observe differences between the decay rates computed for excised porcine skin and live human skin (Table S3, Fig. S10). These differences may be due to the fact that live human skin can absorb the components of the mosquito repellent (including the less volatile ones), while the absorption by excised porcine skin may be slower. Natural thermoregulation and physical movements of the sampled skin surface may also play a role in the volatilization and absorption of the repellent components coating the skin surface.

Taking into account the limits of detection (LODs) of the GC-MS method ($1.34 \times 10^{-7} - 3.20 \times 10^{-6}$ M, Table S2), the size of the sampling probe (4 cm^2)—and assuming maximum transfer (recovery) of the analytes from the skin to the probe and from the probe to the solvent—the LODs of the entire workflow should be in the order of one nanogram. While this is beyond the scope of this study, the above assumptions should be further verified using isotopic tracers. Although no direct comparisons can be made because of major differences between the target analytes and the used techniques, the above figures put the proposed sampling method side by side with some of the other approaches reviewed previously (*cf.* [2]). However, unlike the other methods (*e.g.* Refs. [3–5]), the proposed method has only been used to detect exogenous compounds. Hence, its general usefulness is yet to be verified.

In summary, we showed a simple way of sampling topical exogenous residues from skin surface for chemical analysis by GC. While the principle is similar to that of the solid-phase microextraction using fiber-shaped sorbents [10–12], here a soft flat biocompatible inexpensive material was used to enable efficient sampling from skin surface. Although the presented method can only be regarded as semi-quantitative, it can help to characterize the retention kinetics of skin-care products on the surface of skin. Volatility of natural ingredients (*e.g.* in citronella oil) is a major consideration when developing new formulations of natural skin repellents [13]. The proposed sampling method can support the development of such formulations, and help to achieve the goal of increased retention on the skin surface. Importantly, the oil-control blotting paper is an inexpensive, widely available, and biocompatible material. Further work is warranted to evaluate the compatibility of this sampling approach with other detection systems including on-line atmospheric pressure interfaces of mass spectrometers, to verify the possibility to analyze skin metabolites in addition to xenobiotics, and to include more specimens and tested parameters following the specified guidelines (*cf.* [14]).

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2018.08.008.

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