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Supplementary Materials for

Point-of-care applicable metabotyping using biofluid-specific electrospun MetaSAMPs directly amenable to ambient LA-REIMS

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Text
Supplementary Notes

Supplementary note S1: Initial optimization of electrospun core membranes for biofluid-specific metabolic fingerprinting

S1.1 Evaluation of different polymer solutions for the core membrane of the rectal MetaSAMP®

PVP (MW=360,000 g/mol) and PS (MW=192,000 g/mol) were purchased from Sigma-Aldrich (US). Different ratios and mixtures of polymers were selected, *i.e.,* cross-linked PVP and two blends of PVP and PS (consisting of 20% and 50% of PVP, *w/w*), and investigated as ab- and adsorptive membrane substrates for rectal metabolome sampling and analysis. ES solutions were prepared by dissolving variant amounts (expressed in weight percentages, *w/w*) of PVP and PS in DMF and $CHCl_3$ (1:2, *v/v*) and stirred for a minimum of 4 h at 60°C to obtain a clear solution. A dual bidirectional mono-nozzle in combination with a rotating drum design (20 cm in length and 10 cm in diameter) was used to deposit the nanofibers on aluminium foil (custom-built at Ghent University, Fig. 1C). The morphology of the electrospun membranes was visually inspected through SEM analysis (fig. S1 and see also note S2).

The sampling membranes were impregnated with porcine rectal content and subjected to LA-REIMS analysis to evaluate metabolome coverage as compared to the analysis of crude liquid rectal content. Alterations in the metabolic fingerprints generated upon modification of the composition of the membranes tested were addressed with ANOVA at a confidence interval of 95% (p-value of 0.05 using Bonferroni's correction) and normality evaluation by the Shapiro–Wilk test (p-value>0.05) using IBM® SPSS® 27. In light of LA-REIMS analysis of the rectal metabolome, significant differences were observed for all electrospun blends when compared to crude rectal content analysis, *i.e.,* a significantly higher coverage was observed (in terms of molecular feature count) (ANOVA and Tukey p<0.05, fig. S2). It was however noted that metabolic coverage by PVP/PS 50/50 significantly outperformed all other compositions and samples tested (cross-linked PVP and PVP/PS 20/80 were not significantly different). Consequently, these findings led to further optimization work in finetuning the relative amounts of PVP and PS.

S1.2 Evaluation of different polymer solutions for the core membrane of the salivary and urinary MetaSAMP®s

Polymer solutions for the production of urinary and salivary core MetaSAMP® membranes were prepared as described in note S1.1 and in the Materials and Methods section: Electrospinning. In contrast to the initial optimization phase of the rectal MetaSAMP®, cross-linked PVP was not evaluated as the PVP/PS blends significantly outperformed the hydrophilic homopolymer electrospun membrane regarding metabolome coverage for the rectal MetaSAMP®. Consequently, the focus of the core membrane's development was on the combination of the hydrophilic (PVP) and hydrophobic (PS) polymers. However, because saliva and urine are even more aqueous compared to rectal content and to enhance the ab- and adsorption of its more apolar constituents, 100% PS was additionally electrospun, next to different PVP/PS blends (50, 60, 70, 80 and 90% PS). When spiking the different membranes with saliva or urine, residence and thus impregnation times were unacceptably long (>1 h), *i.e.*, the biofluid drops remained intact on the surface of the membranes. As a result, no reliable LA-REIMS fingerprints could be obtained. The different membrane compositions were also evaluated in terms of fiber size morphology (fig. S5). These analyses clearly demonstrated that a certain fraction (min. 10%) of hydrophilic polymer (PVP in this case) did benefit the nanofiber size distribution. Following these observations and in line with the arguments provided in the main Results section, an additional relatively hydrophilic polymer, PAN, was included both as a cover layer and in the core blend in the second round of optimization (see Results in paper).

S1.3 Rectal MetaSAMP® impregnation time and volume

Different volumes (20-100 µL) of fecal water were spotted onto membrane pieces (1 x 1 cm in size) and subsequently inspected regarding membrane integrity/stability, drying/impregnation time etc. (fig. S15A-B). The electrospun membrane pieces were oversaturated (fluid leaking out) for volumes ≥50 µL, while impregnation time needed to be drastically extended (up to 2h) to have a representative LA-REIMS analysis which did not favor the direct aim of our approach. Consequently, spike volumes of 20, 30, and 40 µL were tested equivalent to applying 2, 3, and 4 droplets in light of the 'at patient's home' application. The latter volumes as well as 2 droplets were ad- and/or absorbed almost immediately after impregnation (fig. S15B) while applying 3 or 4 droplets of fecal water was ad- and/or absorbed completely or only partially after 10 to 15 minutes and over 15 minutes of impregnation, respectively. It was observed that 20 µL and 2 droplets of biofluid were not sufficient to cover the entire membrane surface (small dry spots remained). Also, the membrane pieces impregnated with 20 µL or 2 droplets dried out quickly under ambient conditions, while the

impregnation of 40 µL left the membrane pieces completely wet for the duration of the analysis. The membranes spiked with 30 µL, 40 µL or 3 and 4 droplets of fecal water remained wet longer, although a small accumulation of free liquid was seen on the membranes spiked with 40 µL or 4 droplets. Considering the inevitable sample loss during storage and analysis, we eventually opted to spike the membranes with 3 droplets, equivalenting 30 µL of spiked volume.

To assess the effect of the residence time of the biofluid on metabolome coverage at ambient conditions, 30 µL of the ¼ homogenized fecal slurry was spiked for 15 min or 1 h on the MetaSAMP®s. It was observed that the feature count and repeatability were similar to superior when impregnating 15 min (3,589 features and 78.15% molecular features below CV threshold) instead of 1 h (3,577 features and 34.81% molecular features below CV threshold).

Supplementary note S2: Characterization of the polymer network

Before ES the polymer blends (PVP/PS/PAN), the polymer solutions were cooled to RT (22±2°C), and their viscosity was measured using a volume of 7 mL by the operation of a Brookfield viscometer LVDV–II Pro+ (DE). To enable a qualitative analysis of all electrospun membranes, the morphology of the fibers was examined via SEM analysis using an FEI Quanta 200 FFE (US) at an accelerating voltage of 20 kV. Before SEM analysis, 7 mm sample pieces were cut and coated with platinum using a sputter coater Balzers union SCD 030 (CH). The average fiber diameter was estimated based on 100 fiber measurements per sample and calculated by ImageJ™ (US) version 1.52 processing and analysis software.

Modulated differential scanning calorimetry measurements (mDSC) were performed to determine the glass transition temperatures (Tg) and assess the miscibility of the polymers in the blend. A TA Instruments Q2000 (Waters Corporation, US) was applied, equipped with a refrigerated cooling system and using nitrogen as purge gas (50 mL min^{-1}). The samples were analyzed in aluminium Tzero hermetic DSC pans (TA Instruments), which were loaded with 2.50±0.5 mg fibers. The instrument was calibrated using Tzero technology for standard Tzero aluminium pans using indium at the heating rate applied during measurements. The heating rate was set at 2 K min^{-1} and 5 K min^{-1} and a temperature modulation of ±0.7°C and ±0.8°C every 60 s was selected, for the PVP/PS (60/40, *w/w*) with PAN and the preliminary blends of PVP/PS without PAN cover, respectively. Samples were subjected to two heating cycles from 0°C to 200°C. Tg was determined with TA Universal Analysis software (Waters Corporation, US).

Static contact angles were determined with an OCA 25 contact angle setup from DataPhysics (DE) using a droplet of 2 µL demineralized water or 2 µL fecal water at RT (22±2°C) and RH of 30±10%. Per sample, 10 to 20 measurements were executed. The evolution of the contact angle over time was measured by movies recorded with a frame rate of 8 frames s^{-1}.

Supplementary note S3: LA-REIMS analysis

S3.1 Optimization of LA-REIMS parameters for the rectal MetaSAMP®

In parallel to the optimization of the rectal MetaSAMP®'s core membrane composition, LA-REIMS parameters were inspected using Modde™ (Sartorius, DE) regarding their optimal settings for analysis of impregnated electrospun rectal core MetaSAMP® membranes. Hereto, the influence of six factors was evaluated: the flow rate (150-300 µL min^{-1}), the cone voltage (20-80 V), the heater bias voltage (20-80 V), the scan time (0.1–1 scan(s) s^{-1}), laser ablation time (1-6 s) and the laser pulse energy (165-180 µs). Their effect was statistically evaluated (ANOVA, $p<0.05$) based on the metabolome coverage in terms of the total number of molecular features and the overall signal intensity (fig. S4). The optimal settings were confirmed by assessing the repeatability ($n=10$ membrane pieces) using % of components with a CV ≤30% and can be consulted in table S1.

S3.2 LA-REIMS instrumental analysis specifications

Briefly, daily calibrations of the MS instrument were performed (0.5 mM sodium formate solution in 90:10 IPA: UPW (*v/v*)) to ensure the highest mass accuracy *(1,16).* The analyte vapor was mixed with a constant infusion of IPA in a T-piece unit containing leucine enkephalin (0.25 ng μL^{-1}) as an external calibration compound to correct for potential instrumental mass drift between analytical batches *(1)*. The flow rate (Pump 11 Elite, Harvard Apparatus, US) and other LA-REIMS settings differed according to the methodology addressed, as described by Plekhova *(1)*. In brief, a mid-IR laser system (Nd:YAG laser (operating wavelength of 2.94 µm), Opolette 2940 (Opotek, US) was used to rapidly heat each sample, causing evaporation (IR laser light absorption by water molecules, abundantly present in biological matrices) and partial ionization. A PTFE transmission tubing was placed above the sample surface and served to capture the resulting vapor wherafter the aerosol was transferred further into the MS system (Xevo G2-XS equipped with the REIMS source, Waters Corporation). There, the sample vapor was mixed with the co-infused solvent matrix towards

signal enhancement and collided onto a heated impactor, whereby individual molecular species are generated and transferred to the mass spectrometer additional ionization and analyte declustering took place, as well as mass separation and detection (QToF).

Analyses by LA-REIMS were performed in a randomized order, thereby taking appropriate QC measures to correct for unsystematic variance by running pooled samples before the analysis to achieve adequate instrument conditioning as well as in between every 10 samples to monitor and compensate for possible analytical drift. The *m/z* scan range was set from 50 to 1,200 Da in full scan acquisition mode in negative ion analysis mode with a scan time of 0.3 seconds s^{-1}. Every sample was analyzed at least in technical triplicate (n=3 burns).

S3.3 LA-REIMS set-up specifications

For the crude biofluid analysis, we used our fully automated set-up *(1)*. This is, a volume of 100 µL of biofluid was spiked into a 96-well plate mounted on an automated stage towards remote and automated fingerprinting. For the analysis of MetaSAMP®s, we used a semi-automated approach. This is, the electrospun membrane pieces (1 x 1 cm in size) were placed into a 24-well plate and spiked with 30 µL for which the optimization is described under note S1.3. The well plates were positioned onto the motorized platform for LA-REIMS analysis, but every burn was initiated manually via the software instead of using the automated acquisition pipeline. Before, such manual analysis was done by positioning the sample to be analyzed on glass microscope slides. However, as the MetaSAMP®s dimensions perfectly fit the dimensions of the individual wells of a 24-well plate, we adapted this approach. The different volumes addressed in our manuscript are thus the result of such differences in methodology, including the different well-plates used, as described previously by Plekhova *(1)*. Spiking 20-50 µL into a 24-well plate equals the height positioning of the sample onto microscope glass slides as well as it equals the height of adding 100 µL of a specific biofluid into the wells of a 96-well plate. This height represents an optimal distance between the focusing lens and the sample, resulting in high-intensity signals when ablating the sample using LA-REIMS.

Supplementary note S4: Characterization of the interaction between the electrospun polymer network and laser ablation

In order to assess the interaction between substrate and analytical beam, *i.e.,* the optimized MetaSAMP®s and laser ablation, respectively, the following experiments were

performed using the rectal MetaSAMP® as a representative given its subjection during LA-REIMS analysis to the highest laser energy (table S1). The rectal MetaSAMP® was impregnated with UPW and subjected to ablation with the laser energy that was optimized for the analysis of impregnated rectal MetaSAMP®s (175 µs OPO delay time, table S1 and fig.S16E) or the maximum possible laser energy achievable using our mid-IR system (165 µs OPO delay time, fig S16A) and further compared with impregnated fecal water subjected to same optimal and highest laser energies (fig S16C and S16D, respectively) while monitoring the background IPA signal (fig. S16B) *(1)*. No polymer peaks derived from PVP and/or PS were observed in any of the mass spectra obtained following the ablation of the rectal MetaSAMP® (fig. S16C, D and E). Only when the UPW-impregnated rectal MetaSAMP® with the highest possible laser energy was analyzed, some PAN peaks in the mass range 380-800 Da appeared in the extracted chromatograms (fig. S16A, right). Despite the latter, no PAN, PVP and/or PS peaks were visible in the spectra obtained of our rectal MetaSAMP®s impregnated with fecal water. These experimental results have thus indicated that the mass spectra resulting from rectal MetaSAMP®-LA-REIMS analysis are representative of the impregnated fecal metabolome and hence free of peaks originating from the MetaSAMP®s polymeric constituents.

Additionally, the repeatability of consecutive burns (n=10, impregnated rectal MetaSAMP®s), *i.e.*, membrane stability upon LA-REIMS analysis of one and the same ablation spot, was evaluated. The average number of detected molecular features was 1700 with an RSD of 1%, with an average of 7% of unique features within a single replicate. Regarding those molecular features, 84.1% had a CV ≤30%. The average TIC of the replicates was 5.74 e^5 with an RSD of 9.2% and did not decrease throughout the repeated ablations. These results evinced the metabolome's stability upon LA-REIMS analysis of the same ablation spot of the impregnated rectal MetaSAMP®s, both in terms of feature yield and signal intensity, despite possible damage to the fibrous network.

Hereafter, SEM analysis was performed on all impregnated MetaSAMP®s analyzed as described above. It could be seen from the images where the optimal (lower) laser energy was applied, that the electrospun networks, either impregnated with UPW (fig. S17A) or fecal water (fig. S17C), were only impacted superficially and fibers remained intact. The thickness (electronic digital micrometer 0-25 mm/0.001 mm) of our MetaSAMP®s (n=5) was 86.4±7.20 µm. In contrast, when applying the highest laser energy (165 µs) on the membranes impregnated with UPW and fecal water, the whole membrane was penetrated or collapsed (fig.S17B-D), respectively.

As mentioned by Fatou *(20)*, the laser beam penetration depth is mainly related to the optical absorption coefficient of water molecules at the used laser wavelength (2.94 µm) and is expected to lie within the µm range. However, if addressing the highest energy level possible of the mid-IR system, the fibrous network did collapse (fig. S17B, D), in line with the spectra we recorded that demonstrated PAN peaks for the rectal MetaSAMP® impregnated with UPW (fig. S16A right).

To summarize, analysis of our MetaSAMP®s using the tuned LA-REIMS settings showed no polymer-related degradation peaks nor substantial structural damage to the electrospun fibrous network.

Supplementary note S5: Biological sample selection for rectal MetaSAMP® impregnation with pediatric cohort fecal samples

To select a representative human fecal sample for impregnation, fecal slurry, ½-, ⅓- and ¼-dilution of the fecal slurry (termed fecal water), and lipid, as well as aqueous extract, were prepared, according to our optimized lipidomics and metabolomics procedures *(11, 77)*. For the representative fecal sample, the lipid extracts clustered separately from the other fecal preparations, indicating the least multivariate similarity in its metabolic fingerprint (fig. S19). Also, the PCA model and CV calculations revealed repeatability of LA-REIMS analysis of undiluted fecal water was lower than that of diluted fecal samples (table S13). The decreased repeatability and molecular feature count of the undiluted fecal sample could be a result of saturation and too little presence of water to have an efficient desorption and ionization process. A plausible reason for the tight clustering of the polar extracted samples together with the fresh fecal samples and dilutions thereof as opposite to the lipid extracts could be sought in the respective extraction protocols. The polar protocol is less intensive than the one of lipidomics, which also uses methyl-tert-butyl ether, among others, as an extracting agent. Besides, the sample pretreatment in polar metabolomics is mainly focused on the precipitation of proteins. The stomaching procedure for the preparation of the fecal water samples involved a high-intensity mixing process and centrifugation, which, together with additional dilution by UPW, may have resulted in significant protein precipitation and as such similar metabolome fingerprints to the ones observed in the polar extracts. Besides, the use of extracts involves extensive sample pretreatment steps which are in great contrast with the direct workflow aimed at. The PCA indicated few outliers (95% Hoteling's T^2 statistics) which could follow the inherent variation, *e.g.,* the amount of water, in feces which was corrected for upon dilution of

the fecal samples (2-, 3- and 4-fold dilution) and through the execution of streamlined polar metabolomics and lipidomics protocols. Given best repeatability (lowest CV and highest % of molecular features with CV below the threshold of 30%, table S13) without significant decrease in molecular feature count and tight clustering of the fecal fingerprints (fig. S19) was observed for the most diluted fecal sample, the 4-times diluted fecal water was chosen.

To provide evidence of the binding of the analytes to the fibers (*i.e.,* prove their ab- and adsorptive effect), the recovery was assessed for a selection of metabolites (n=16, based on tables S7-S9 and 7 internal standards) in a targeted manner by performing a generic extraction of our MetaSAMP® substrates and consecutive analysis through hyphenated UHPLC-HRMS. To this end, impregnated rectal MetaSAMP®s (30 µL of the ¼ homogenized fecal slurry (100 mg feces, 300 µL UPW) and fecal slurry (100 mg feces, 300 µL UPW)) (n=5, pooled QC sample (n=6) were spiked with a mixture of internal standards (10 µL, n=7, 2 ng μL^{-1}). Next, the endogenous fecal metabolites and spiked internal standards were recovered using a generic metabolomics extraction protocol. Here, 100 µL of a mixture of ice-cold MeOH and UPW (80/20, *v/v*) was added to the center of the impregnated membranes and to the crude feces in line with *(11)*. The membranes were impregnated for 15 min and the supernatant was collected, while the feces samples were vortexed, and centrifuged (for 5 min at 13,000 g) after which the supernatant was also collected. Both extracts were analyzed by UHPLC-HRMS analysis as described previously *(11)*. The recovery was calculated as the area ratio between the integrated peak area of each metabolite detected in the membrane extract or the feces extract, both normalized by deuterated internal standards for each metabolite *(83)*, since they can be considered as pre- and post-spiked, respectively. Overall, recovery percentages were >100% for the majority of metabolites (12 out of 16) (table S10).

Subsequently, the linearity (untargeted and targeted) of the LA-REIMS fingerprinting method was assessed by setting up an 8-point fecal water dilution series (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8) (pooled QC sample, n=6) in quintuplicate (n=5) both directly followed by LA-REIMS analysis and after impregnation on the rectal MetaSAMP®. The determination coefficient (R^2) was obtained for each molecular feature detected in the dilution series, and the number of molecular features with $R^2 \geq 0.7$, 0.8, and 0.9, respectively calculated. In addition, the fecal slurry was spiked with standard mixtures (table S7) at 3 concentrations: 33, 50, and 100 ng μL^{-1}, allowing targeted linearity evaluation of each metabolite through a 4-point dilution series (including fecal water without the standard mixtures added as a blank) by calculating R^2. We found that the number of molecular features detectable in all dilutions tested showed an improved linear response in the MetaSAMP®-LA-REIMS analysis compared to the LA-

REIMS analysis of crude feces, while most of the targeted metabolites (5 out of 8) had an R^2 ≥0.9 (table S11).

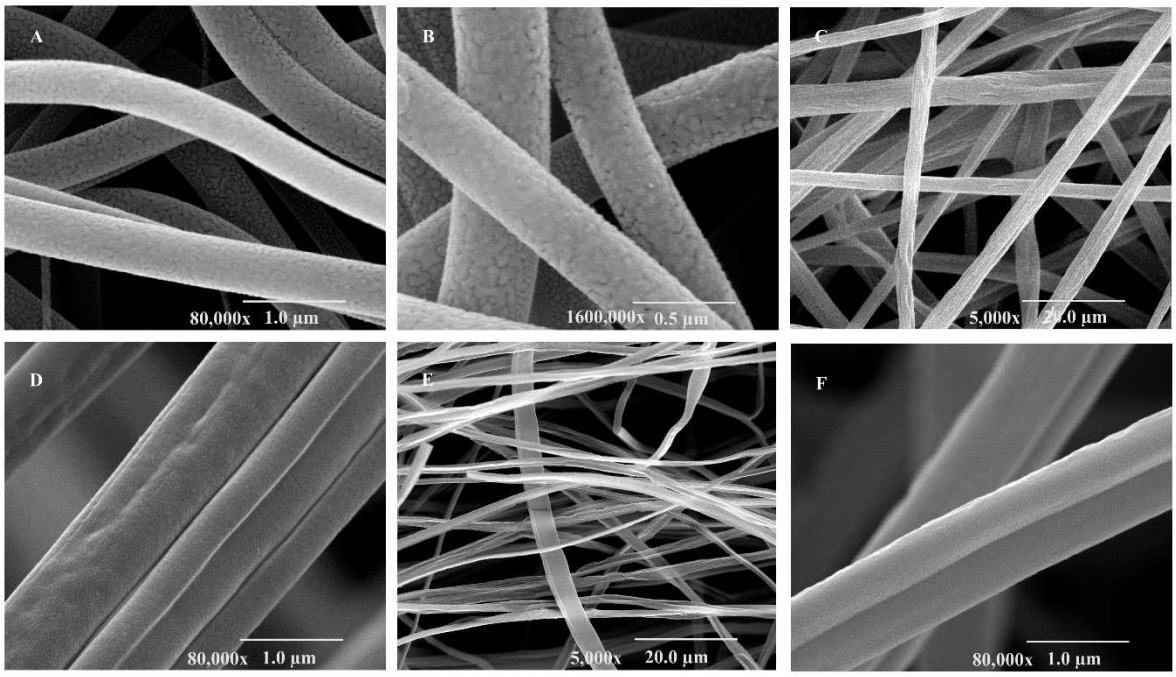

Fig. S1.

SEM images of PVP and PS blends demonstrate nanofibrous structures.

(A, B) 100% cross-linked PVP, showing homogenous nanofibers which are composed of one polymer type. **(C, D)** and **(E, F)** 20/80% and 50/50% PVP/PS, respectively, displaying a microporous and homogeneous (*i.e.,* the 2 polymers thoroughly mixed) polymer network. The different superficial fiber structures shown in **(B, D, F)** could be ascribed to the evaporation rate of the solvent system (DMF/$CHCl_3$, 1:2, *v/v*) that varies upon the electrospinning of 1 or 2 (mixed) polymer type(s), respectively.

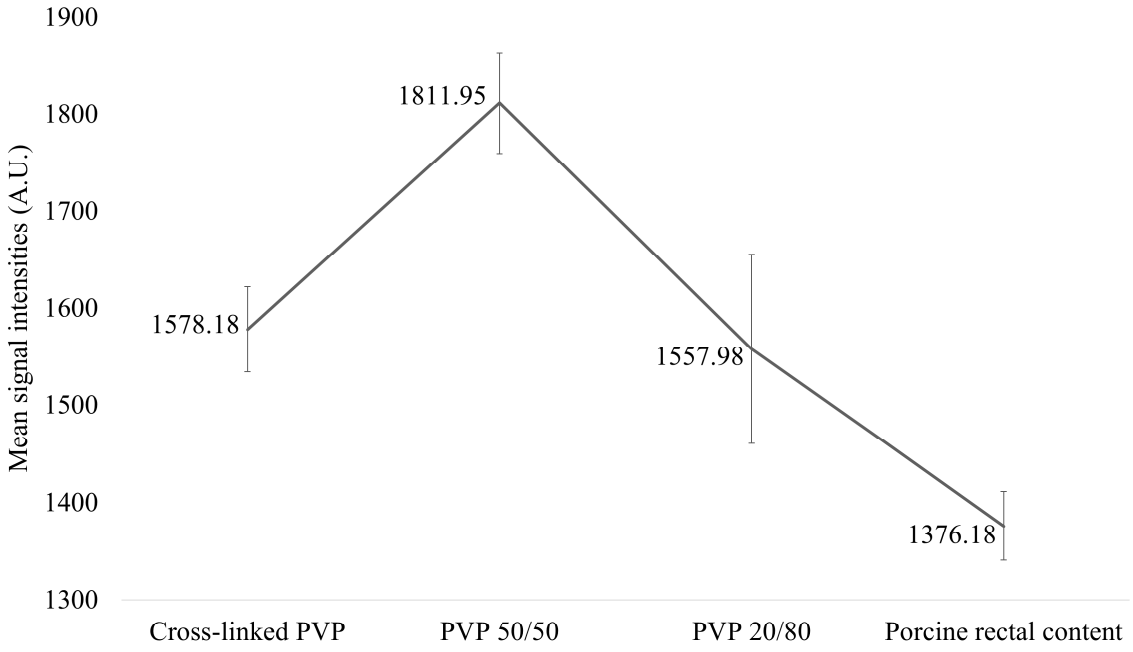

Fig. S2.

Comparison of different electrospun compositions with porcine rectal content.

Mean plot (ANOVA, $p<0.05$) illustrating the mean normalized signal intensities and their standard deviation ($n=5$) following LA-REIMS analysis of different compositions of impregnated (porcine rectal content) nanofibrous sheets compared to LA-REIMS analysis of crude porcine rectal content.

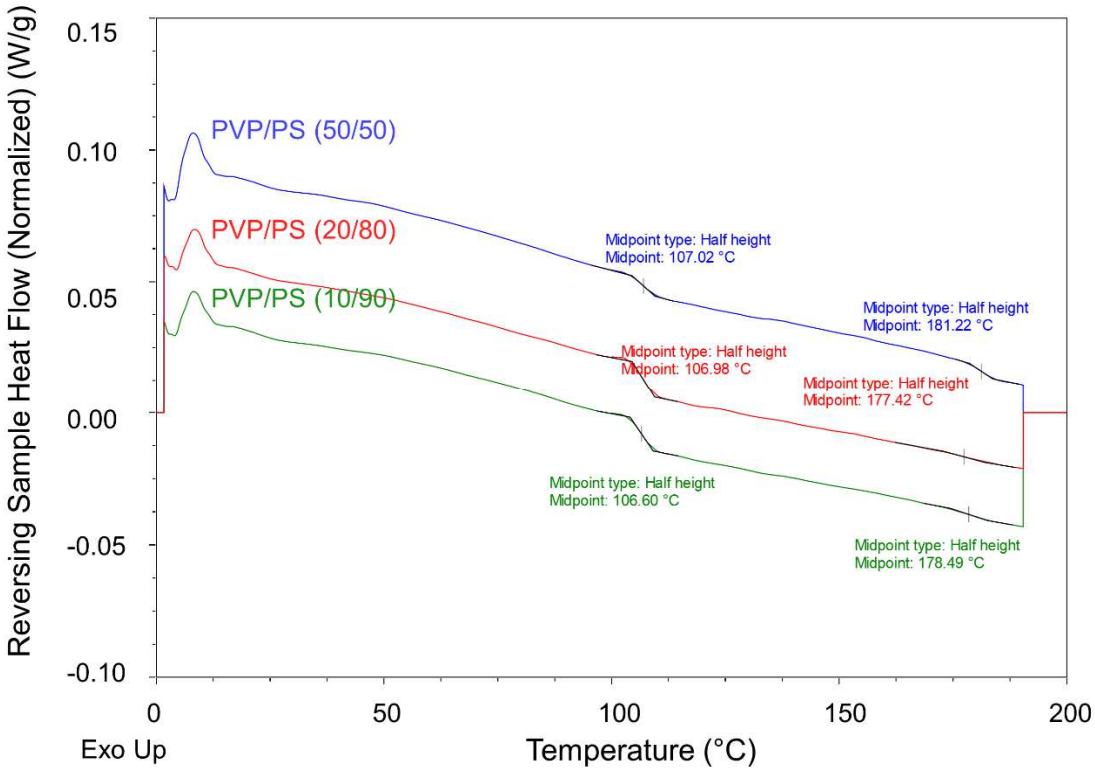

Fig. S3.

MDSC measurements of different electrospun compositions.

Tg values are depicted as the inflection point of the indicated transitions of test blends of PVP and PS (consisting of 10, 20, and 50% PVP, *w/w*).

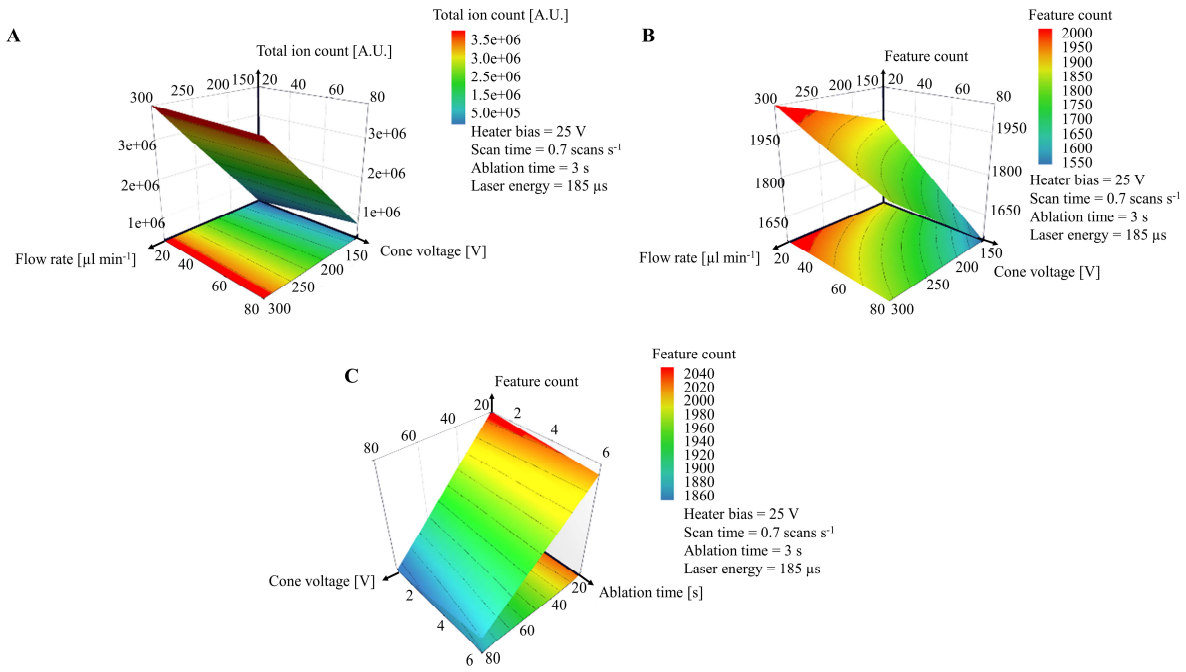

Fig. S4.

Three-dimensional contour plots of the optimization of the LA-REIMS settings for the analysis of the rectal MetaSAMP®s.

RSM models showing the effect of the flow rate (150-300 µL min^{-1}), the cone voltage (20-80 V), the heater bias voltage (20-80 V), the scan time (0.1–1 scan(s) s^{-1}), laser ablation time (1-6 s) and the laser pulse energy (165-180 µs) as continuous factors on **(A)** the molecular feature signal intensity (TIC) and **(B, C)** the feature count for the rectal MetaSAMP® impregnated with fecal water.

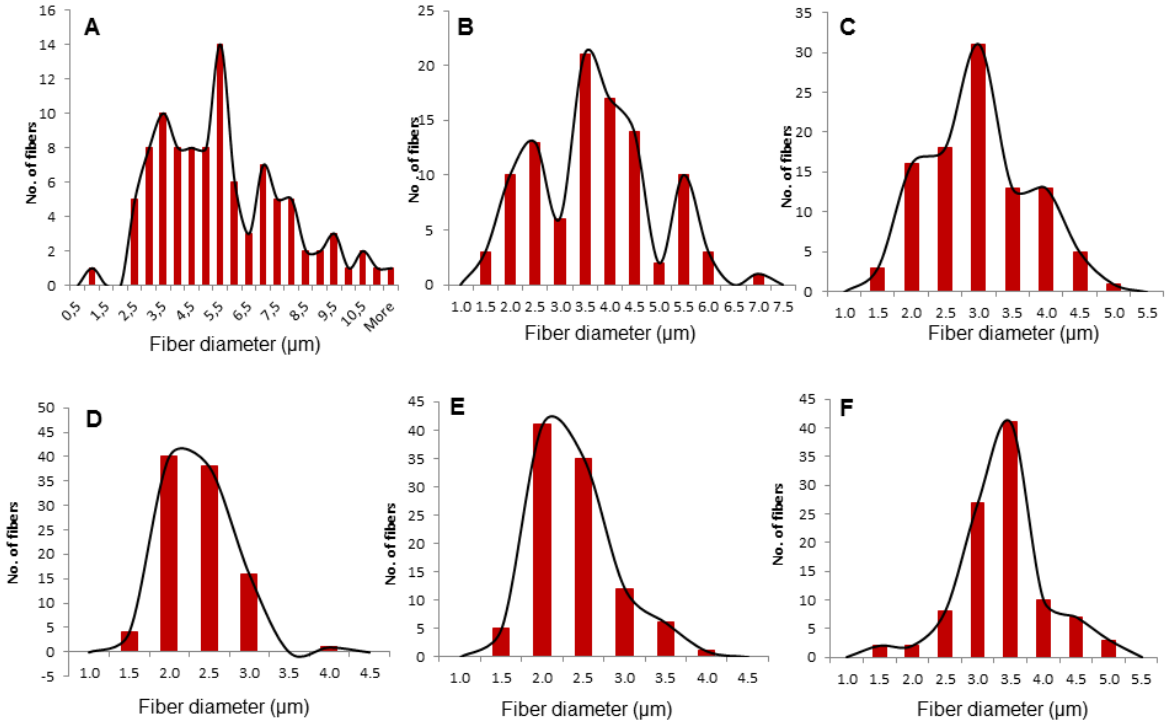

Fig. S5.

Fiber size distributions of membranes electrospun with different PVP/PS ratios.

(A) PS 100%, **(B)** PS 90%, **(C)** PS 80%, **(D)** PS 70%, **(E)** PS 60% and **(F)** PS 50%. Fiber diameter was calculated by ImageJ™ software (*n*=100).

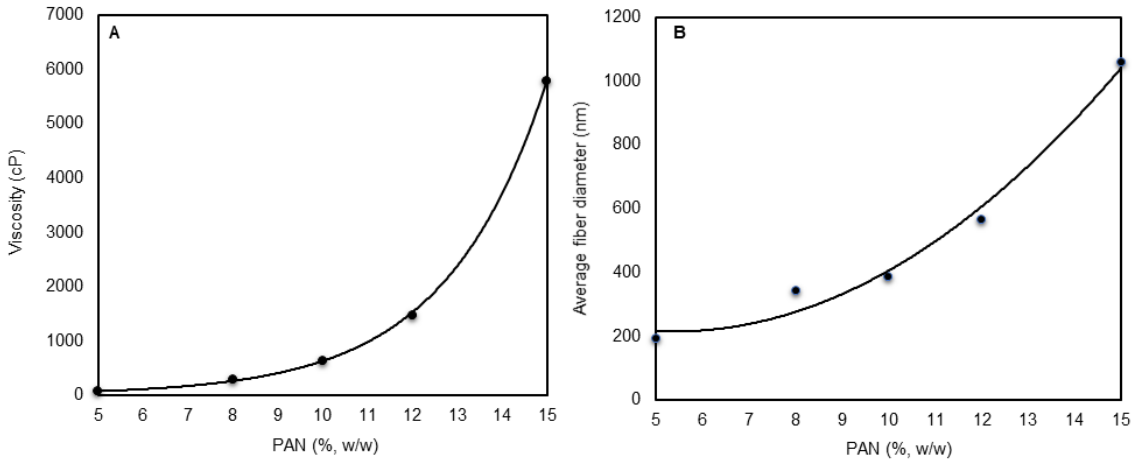

Fig. S6.

Evaluation of various PAN solutions.

(A) Viscosity measurement and **(B)** average fiber diameter of different concentrations (5, 8, 10, 12, 15%, *w/w*) of PAN in solution.

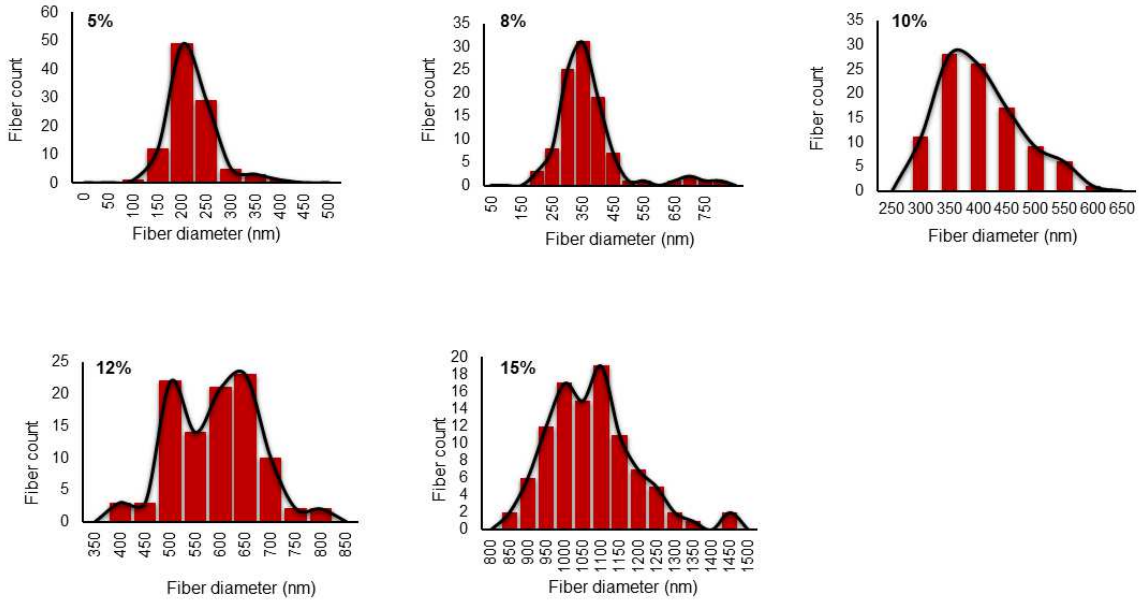

Fig. S7.

Fiber size distribution curves of the PAN cover layer.

Fiber diameter was calculated by ImageJ^{TM} software (n=100).

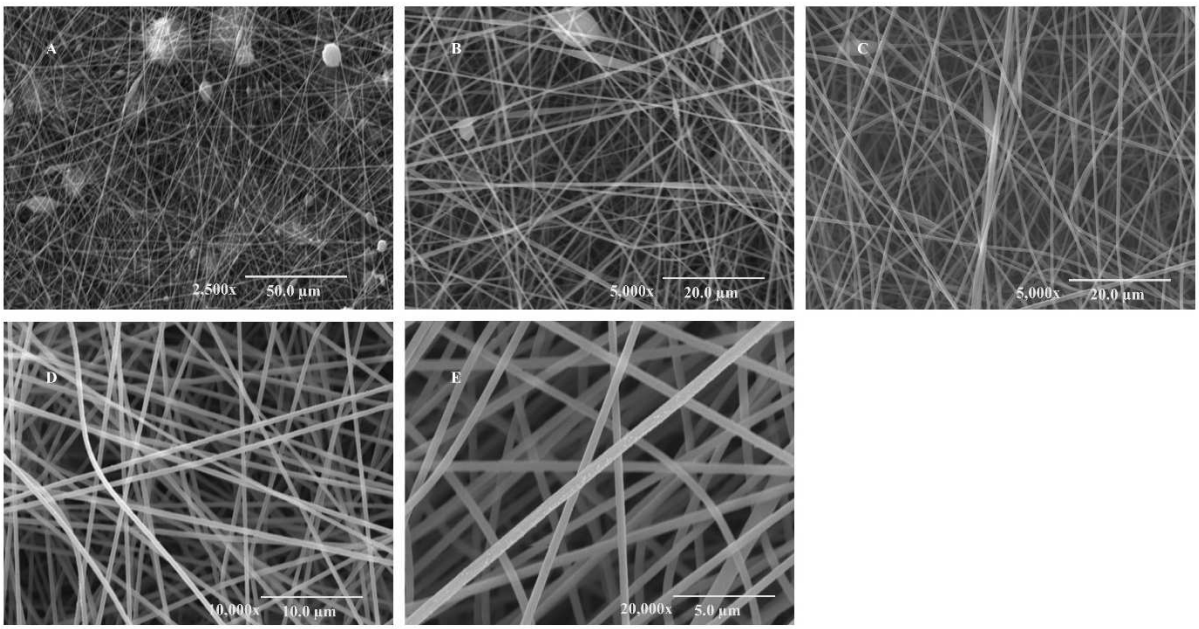

Fig. S8.

SEM images of the PAN cover layer.

(A) 5, **(B)** 8, **(C)** 10, **(D)** 12, and **(E)** 15% PAN (*w/w*). All the membranes were electrospun at 1 mL h^{-1}, 17 kV, and 15 cm tip-to-collector distance.

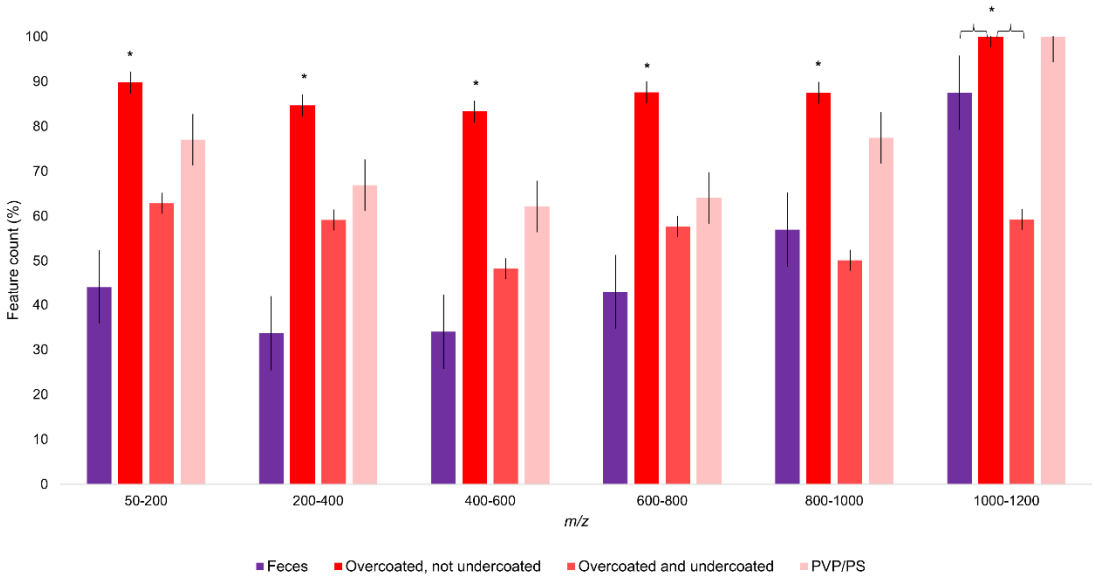

Fig. S9.

Comparison of different positions of the PAN layer.

Graph visualizing the percentage of molecular features with CV ≤30% obtained following LA-REIMS analysis as a measure of repeatability across mass ranges (covering *m/z* 50-1,200 in Da). The sample with merely a PAN cover layer was significantly better or at least as good as the PVP/PS (50/50, *w/w*) blend without a PAN layer across mass ranges but significantly better (p<0.05 indicated with an asterisk) when compared to using PAN simultaneously as both a cover layer and underlayer, and the analysis of fecal water as such.

Fig. S10.

Evaluation of fecal water spreading on rectal MetaSAMP® membrane pieces.

1 x 1 cm membrane pieces were evaluated **(A)** with and **(B)** without a PAN cover layer on top of the PVP/PS (60/40, *w/w*) membranes impregnated centrally with 30 µL fecal water for 1 h before LA-REIMS analysis.

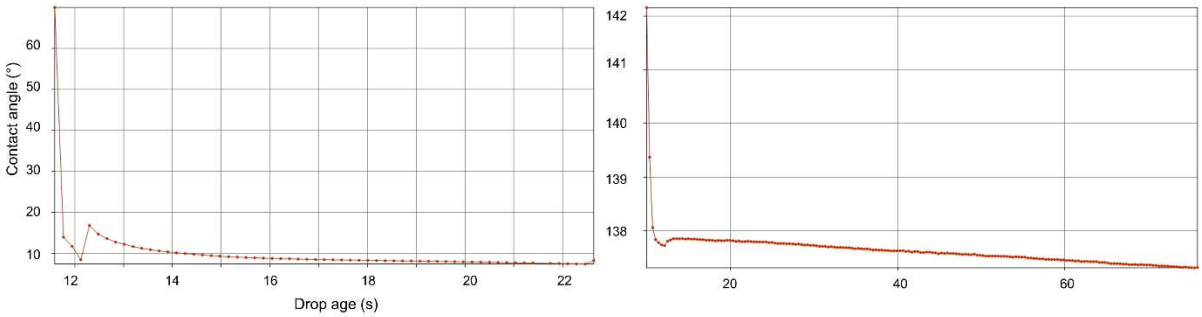

Fig. S11.

Contact angle measurements.

Curves displaying the evolution of the contact angle (°) when fecal water was spotted onto the rectal MetaSAMP® membrane piece with (left) and without (right) a PAN cover layer on top of the PVP/PS (60/40, *w/w*) membranes.

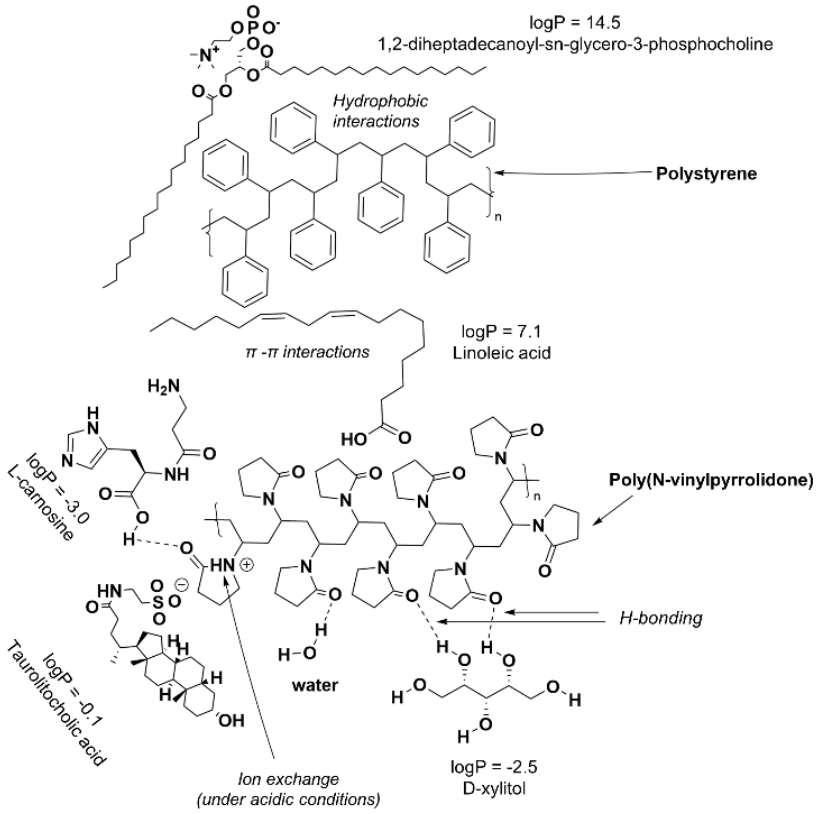

Fig. S12.

Analyte interaction potential with the core PVP/PS layer.

Schematic illustration of the possible chemical and physical interactions that the hydrophobic (PS) and hydrophilic (PVP) polymeric moieties in the nanofibrous sheet may exert towards a selection of metabolites of different polarity. For example, hydrogen bonding, ionic (cation and/or anion exchange), hydrophobic (π-π interactions), Van der Waals and dipole-dipole interactions as well as London forces are amongst such specific interactions.

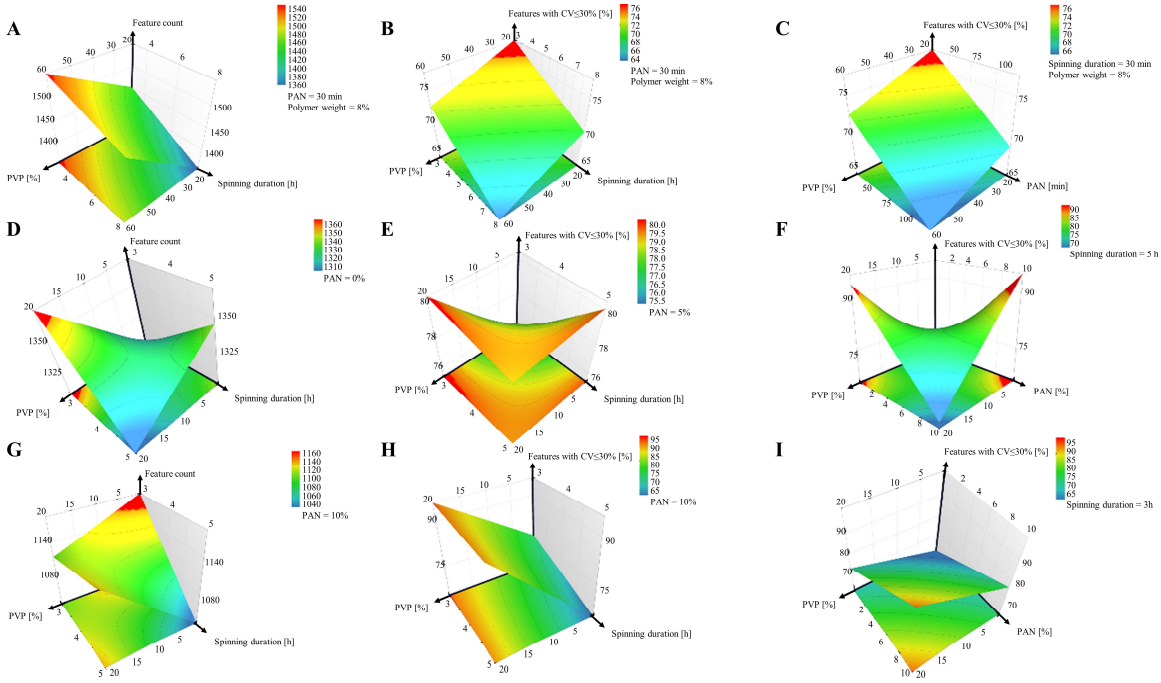

Fig. S13.

Three-dimensional contour plots of the optimization of the biofluid-specific MetaSAMP®'s two-layered compositions.

RSM models displaying the effect of PVP % in the core layer, and/or core layer spinning duration (h), and/or PAN cover spinning duration (min), and/or PAN % in the core layer as continuous factors on **(A, D, G)** the metabolome coverage (feature count) and **(B, C, E, F, H, I)** repeatability (% of molecular features with CV ≤30%). **(A-C)** Rectal MetaSAMP® with 60% PVP and 3h of spinning the core layer, together with 30 min of spinning the PAN cover layer gave the best coverage and repeatability. **(D-F)** Salivary MetaSAMP® showed better repeatability with 3h spinning duration and better metabolome coverage and repeatability with the highest (20%) and lowest PVP (0%), respectively, justifying the compromise of 10% PVP in the core membrane, also because of the more stable fiber distribution at 10% PVP. **(G-I)** Urinary MetaSAMP® provided the best repeatability with 20% PS and 10% PAN in the core membrane layer with no effect of spinning duration, so 3h (shortest) was selected. The parameter altered is depicted at the end of its corresponding axis (black arrowhead).

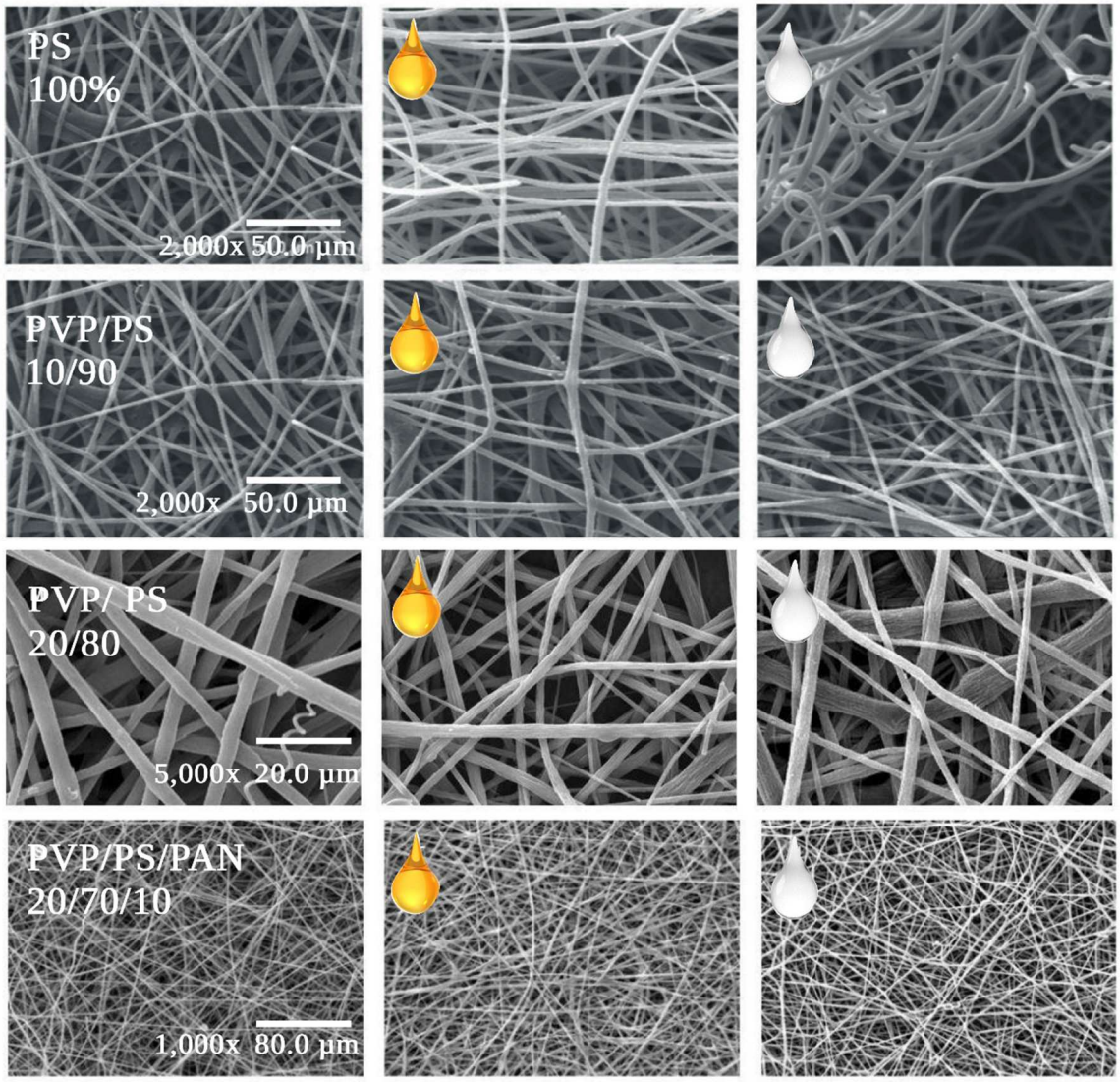

Fig. S14.

SEM images of the core electrospun membranes of the urinary and salivary MetaSAMP®s prior to (left) and following exposure to urine (yellow drop) and saliva (white drop), respectively.

The compositions include 100, 90, and 80% PS (0-20% PVP) and PVP/PS/PAN (20/70/10) blends. The PAN cover layer was carefully peeled off prior to SEM.

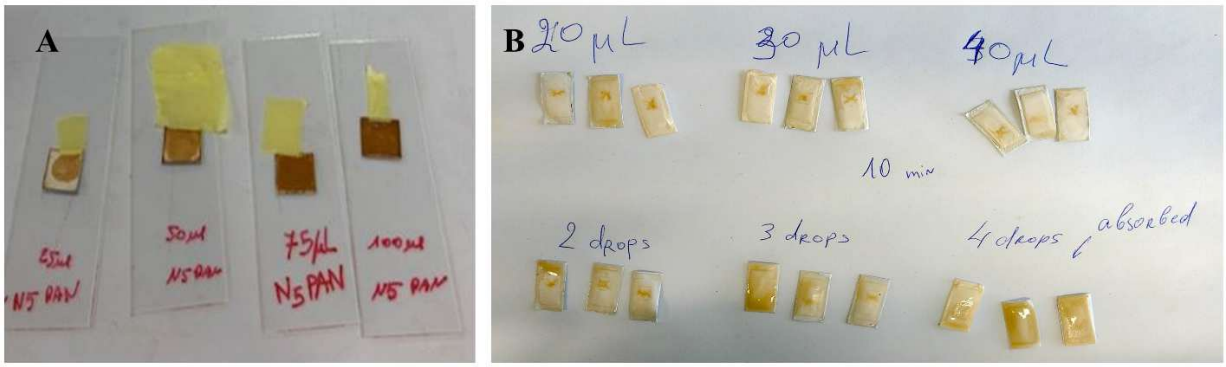

Fig. S15.

Different spike volumes impregnated onto the rectal MetaSAMP®.

Photographs of rectal MetaSAMP®s **(A)** impregnated with 25, 50, 75, and 100 µL of fecal water, **(B)** impregnated with 20, 30, and 40 µL or 2, 3, and 4 droplets of fecal water.

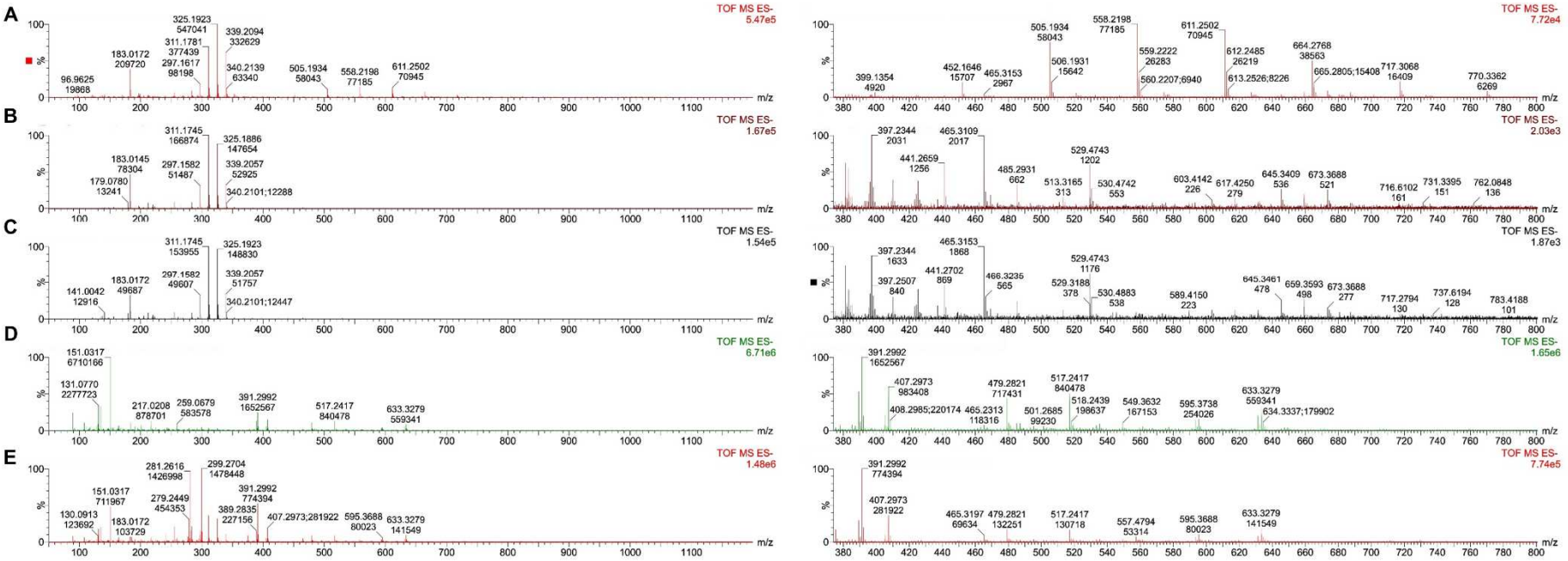

Fig. S16.

Mass spectra acquired from rectal MetaSAMP®s to evaluate the interaction between the electrospun polymer network upon laser ablation. Mass spectra of the entire acquired mass range (50-1,200 Da, left) and a close-up of the 380 to 800 Da (right) mass range for **(A)** rectal MetaSAMP® impregnated with UPW and ablated at the maximal laser energy (165 µs), **(B)** pure IPA background signal (infused solvent matrix), **(C)** rectal MetaSAMP® impregnated with fecal water and ablated at the optimal laser energy (175 µs), **(D)** rectal MetaSAMP® impregnated with fecal water and ablated at the maximal laser energy (165 µs) and **(E)** rectal MetaSAMP® impregnated with UPW and ablated at the optimal laser energy (175 µs). Several *m/z* peaks corresponding to the PAN polymer (505.1934 Da, 558.2198 Da, 611.2502 Da) could be detected in the membrane impregnated with UPW after laser ablation with maximal energy (165 µs). These peaks are absent from the background or signal obtained from MetaSAMP® impregnated with fecal water.

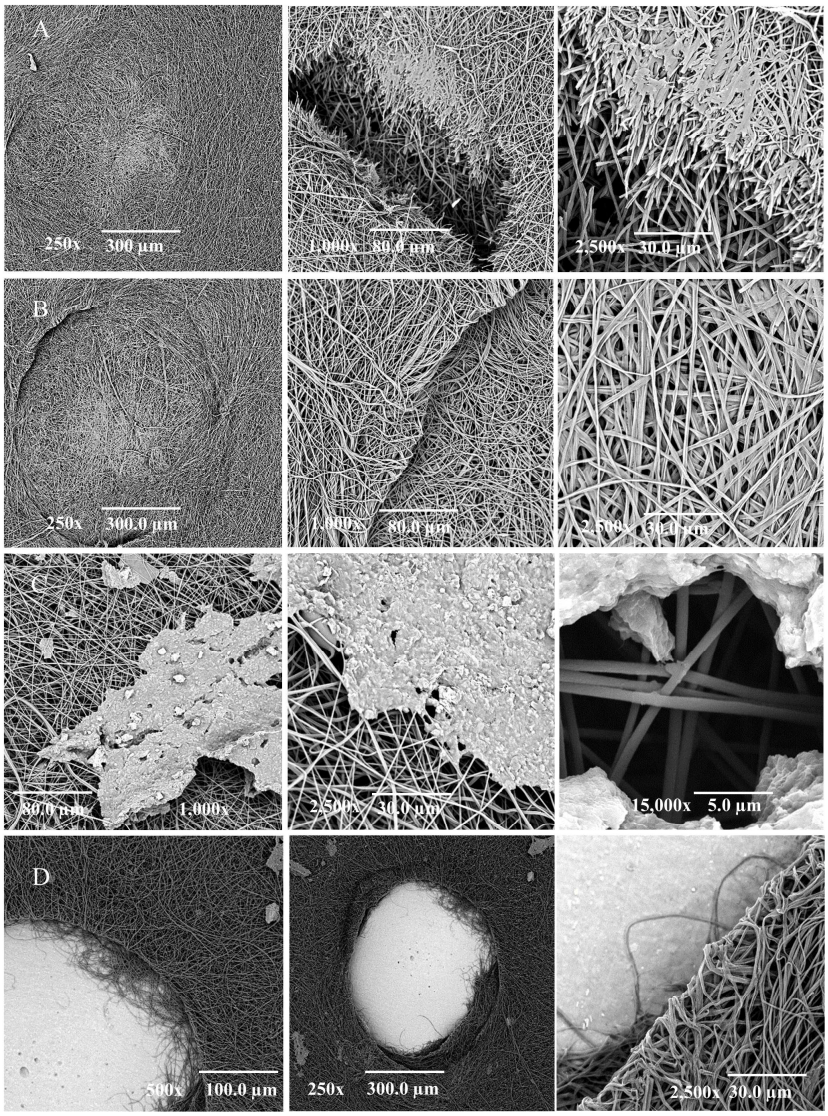

Fig. S17.

SEM images of rectal MetaSAMP®s to evaluate the interaction between the electrospun polymer network and the laser upon ablation.

Rectal MetaSAMP®s were impregnated with **(A, B)** UPW, applying optimal (175 µs) and high (165 µs) laser energy, respectively, and with **(C, D)** fecal water (white concentrated spots), applying optimal (175 µs) and high (165 µs) laser energy, respectively.

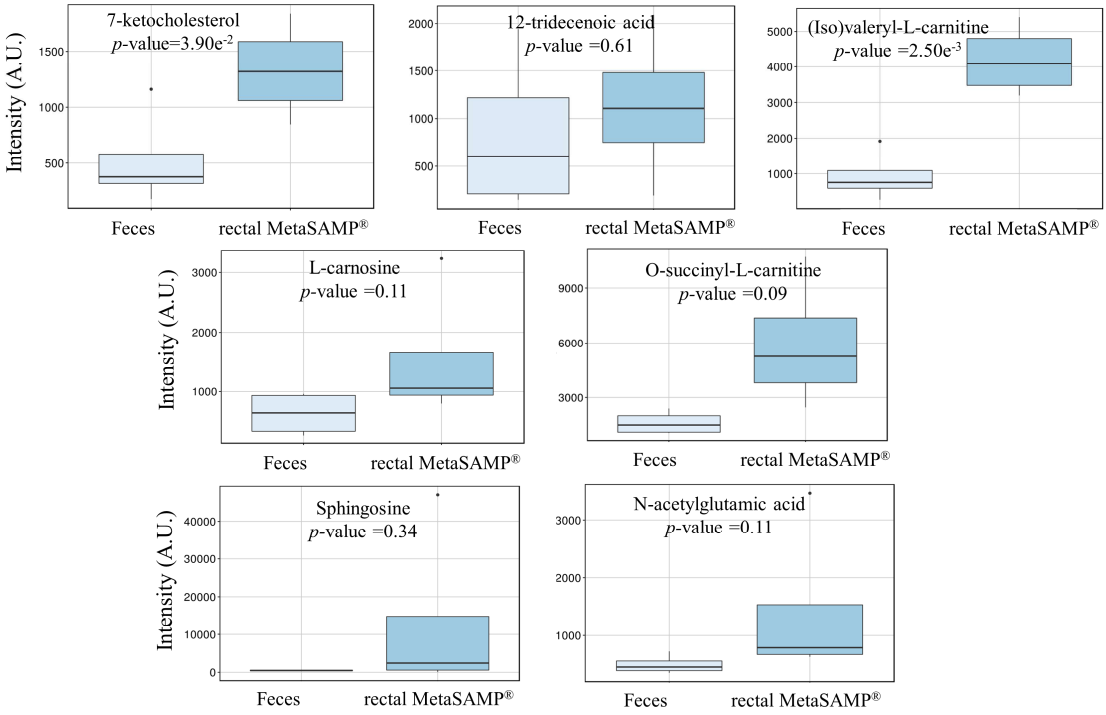

Fig. S18.

LA-REIMS evaluation of the annotated metabolites in crude feces *vs.* rectal MetaSAMP®.

Boxplots of the annotated metabolites comparing (Welch two-sample t-test or Wilcoxon rank-sum test) intensity values upon rectal MetaSAMP®-LA-REIMS compared to crude feces analysis. The interior horizontal line represents the median value, lower and upper bounds of the box represent the 25th and 75th percentile values, respectively, and whiskers are drawn from the corresponding box boundary to the most extreme data point located within the box bound±1.5×interquartile range. The y-axis of the graphs shows the observed intensity for every adduct that was detected in replicated burns, while the x-axis shows the groups compared.

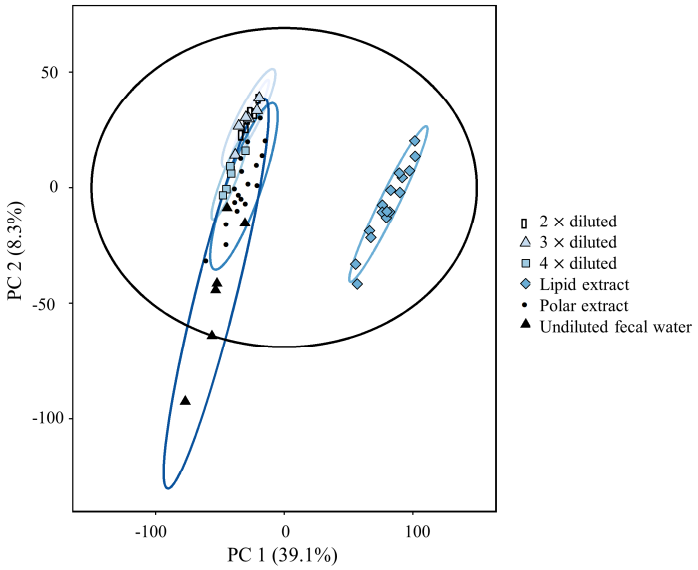

Fig. S19.

Evaluation of different fecal sample preparations.

PCA score plot as obtained upon LA-REIMS analysis of feces that have been pretreated differently.

Table S1.

Optimized Waters Xevo G2-XS QToF instrument operation combined with MID-IR laser (Q-switch delay time) parameters for the analysis in negative (-) ion polarity mode.

The parameters were tuned for the analysis of crude feces *(1)* and the rectal MetaSAMP® when continuously infusing IPA, as well as for the urinary and salivary MetaSAMP®s.

	Fecal water	**Rectal MetaSAMP®**	**Urinary MetaSAMP®**	**Salivary MetaSAMP®**
Polarity	**-**	-	-	-
Solvent flow rate, (µL min^{-1})	150	250	300	200
Cone voltage (V)	70	80	25	45
Heater bias (V)	40	50	25	70
Laser wavelength (µM)	2.94	2.94	2.94	2.94
Laser Q-switch delay time (µs)	180	175	185	180
Pulse ablation time (s)	3	3	3	3
Scan time (scans s^{-1})	0.3	0.7	0.5	0.5

Table S2.

Effect of various PAN concentrations on viscosity, fiber diameter and distribution range.

PAN concentration is expressed as weight of the polymer to the total solution weight (*w/w*).

PAN (%, *w/w*)	Viscosity (cP)	Average PAN fiber diameter (nm)	Fiber size distribution range (nm)
5	63.1	192.00	50-450
8	286.5	340.71	150-800
10	625.7	384.00	300-600
12	1,470.5	565.76	400-800
15	5,794.6	1,059.84	800-1,450

Table S3.

Comparison of untargeted metabolome characteristics between feces and the different rectal MetaSAMP® compositions (covering 50-1,200 Da).

Different compositions based on the position of the PAN electrospun layer were evaluated in terms of molecular feature count and repeatability. The compositions comprise a PVP/PS membrane with a PAN cover layer, a PVP/PS (60/40, *w/w*) membrane with both a PAN cover layer and a PAN underlayer electrospun for 15 min. The latter was also done while ES PAN for 30 and 45 min. Additionally, PVP/PS without a PAN layer was also included in the comparison. The corresponding compositions which significantly outperformed the analysis of crude feces *(*ANOVA p=0.004, F=6.63 and df=5 regarding signal intensity and ANOVA p<0.001, F=208.99, df=5 regarding metabolome coverage) were indicated with an asterisk.

Membrane type	Summarised normalized ion intensity±SD	Molecular feature count±SD	% of molecular features with CV ≤30%
Feces	699±37.65	1,457±18.50	53
PAN cover layer*	769±24.69	1,784±5.93	86
PAN cover layer and underlayer (15 min)	728±73.27	1,744±16.82	44
PAN cover layer and underlayer* (30 min)	764±16.61	1,798±16.57	57
PAN cover layer and underlayer* (45 min)	839±22.35	1,798±20.55	59
No PAN*	690±14.07	1,770±14	69*

Table S4.

Contact angle measurements.

The contact angles and SD of nanofibrous membranes with various treatments were measured, illustrating the increased wettability by the addition of a PAN cover layer.

Membrane composition (PVP/PS 60/40, *w/w*) with (+) or without (-) a PAN cover layer	**Treatment**	**Contact angle measured (°±SD)**
+	UPW	89±1
-	UPW	138±1
+	Fecal water	50±2
-	Fecal water	144±4

Table S5.

D-optimal experimental design for optimization of the multilayered composition of the biofluid-specific MetaSAMP®s.

Core layer ES duration (3–8 h), duration of ES the PAN cover layer (30–120 min), PVP (20–60%, *w/w*), and total polymer weight (8–12%, *w/w*) were the factors under evaluation, resulting in 19 experiments, including 3 center points. The average fiber diameter of the PVP/PS network and PAN layer, SEM quality of fiber morphology as well as the number of molecular features and their repeatability were the responses under evaluation. An asterisk was added if the effect of the factor was significant (rectal: ANOVA FDR-adjusted p=0.017, F=13.95, df=19; urine: FDR-adjusted p=0.006, F=2.56, df=19 and saliva: FDR-adjusted p<0.001, F=5.97, df=19) for the number of molecular features. No significant lack-of-fit was observed using Fisher's F-test (95% confidence interval, p>0.05), indicating the models fitted the data and assessed responses well.

Run	ES PVP/ PS (h)	ES PAN (min)	PVP (%, *w/w*)*	Polymer weight (%, *w/w*)	Average Diameter (PVP/PS)† (nm) *n*=100	Number of molecular features (rectal*/urine*/saliva*)	% of features with CV ≤30% (rectal/urine/saliva)	SEM quality (morphology of the PVP/PS blend)†
1	3	30	20	8	1,366 (±300)	1,408/1,359/1,760	73.84/84.25/78.24	Beads
2	8	30	20	8	900 (±200)	1,347/1,409/1,625	75.37/63.09/79.38	Beads
3	3	120	20	8	839 (±210)	1,557/1,461/1,629	75.20/83.98/70.60	Beads
4	8	120	20	8	1,315 (±310)	1,354/1,350/1,545	66.25/62.96/62.85	Beads
5	3	30	60	8	1,118 (±270)	1,561/1,410/1,705	82.38/83.83/61.29	Homogeneous
6	8	30	60	8	1,298 (±250)	1,492/1,441/1,706	57.24/63.80/61.72	Homogeneous
7	3	120	60	8	1,576 (±350)	1,494/1,357/1,622	54.89/58.81/58.26	Homogeneous
8	8	120	60	8	1,726 (±380)	1,436/1,381/1,492	76.01/60.17/44.10	Homogeneous
9	3	30	20	12	Unstable	1,456/1,431/1,551	74.55/75.54/71.50	Beads
10	8	30	20	12	Unstable	1,350/1,347/1,705	65.73/67.93/69.15	Beads
11	3	120	20	12	Unstable	1,437/1,422/1,569	65.99/62.02/58.32	Beads
12	8	120	20	12	Unstable	1,412/1,471/1,761	71.67/75.19/66.14	Beads
13	3	30	60	12	1,626 (±340)	1,491/1,425/1,684	62.66/64.98/64.13	Homogeneous
14	8	30	60	12	1,584 (±330)	1,508/1,434/1,598	75.23/66.88/53.63	Homogeneous
15	3	120	60	12	2,068 (±370)	1,548/1,390/1,679	71.93/64.10/73.38	Homogeneous
16	8	120	60	12	2,151 (±380)	1,448/1,401/1,588	62.52/75.95/56.86	Homogeneous
17	5.50	75	40	10	1,854 (±280)	1,552/1,396/1,648	72.42/63.90/58.19	Homogeneous
18	5.50	75	40	10	2,013 (±340)	1,463/1,428/1,556	60.85/77.17/55.07	Homogeneous
19	5.50	75	40	10	1,821 (±300)	1,518/1,399/1,621	70.45/73.20/69.15	Homogeneous
Feces	NA	NA	NA	NA	NA	1,336	71.83	NA
Saliva	NA	NA	NA	NA	NA	1,517	84.38	NA
Urine	NA	NA	NA	NA	NA	1,377	96.15	NA

†The steady-state stability of the ES process was evaluated visually by observing the Taylor cone in time and by inspecting the nanofibers via SEM in the presence of droplets and beads. The stability was dependent on solution parameters a.o., including polymer ratio and weight (*w/w*), which were varied in the DOE approach.

Table S6.

Experimental design for the optimization of the multilayered composition of the urinary and salivary MetaSAMP®s.

Core layer ES duration (3 or 5 h), duration of ES the PAN cover layer (30 min, unless for the PVP/PS 20/70 composition in which PAN 10% (*w/w*) was added to the blend), PVP (*w/w*) (0–20%) of the polymer blend, and total polymer weight (12%, *w/w*) were the factors under evaluation, resulting in 8 experiments. The viscosity of the various polymer solutions was measured. The average fiber diameter of the PVP/PS network and PAN layer, SEM quality of fiber morphology as well as the number of molecular features and repeatability were the responses under evaluation. An asterisk was added if the effect of the factor was significant for at least one response under evaluation (ANOVA FDR-adjusted $p<0.05$ and Tukey post-hoc $p<0.05$).

Run	ES duration PVP/PS (h)	PVP* (%, *w/w*)	PAN in the blend (%, *w/w*)	Viscosity (cP)	Average diameter (PVP/PS) (nm) *n*=100	Number of molecular features* (urine/saliva)	% of features with CV ≤30%* (urine/saliva)	SEM quality (PVP/PS)
1	3	20	10	532.63	2,120 (±300)	1,114/1,298	96.62/66.44	Homogeneous
2	5	20	10	532.63	2,120 (±300)	1,143/1,330	92.83/67.31	Homogeneous
3	3	20	0	341.20	2,730 (±700)	1,087/1,350	73.14/85.62	Homogeneous
4	5	20	0	341.20	2,730 (±700)	1,121/1,278	87.51/85.77	Homogeneous
5	3	10	0	250.23	3,430 (±1,100)	1,137/1,369	67.37/95.16	Homogeneous
6	5	10	0	250.23	3,430 (±1,100)	1,113/1,367	60.03/90.24	Homogeneous
7	3	0	0	159.93	5,350 (±2,200)	1,133/1,286	63.12/52.00	Beads
8	5	0	0	159.93	5,350 (±2,200)	1,116/1,326	65.76/59.98	Beads
Urine	NA	NA	NA	NA	NA	1,159	97.19	NA
Saliva	NA	NA	NA	NA	NA	1,140	85.55	NA

Table S7.

LA-REIMS analysis of analytical standards in solvent.

The analytical standards were evaluated for their detectability and their measurement characteristics. Mass deviation and accuracy measured in MassLynx™ refer to raw acquired data, respective measurements in Progeneis® QI depict values after processing (peak picking and mass drift correction).

Standard ([M-H]⁻ adduct)	Log P	Measured *m/z* (Masslynx™)	Measured *m/z* (Progenesis® QI)	Mass accuracy (ppm) (Masslynx™)	Mass accuracy (ppm) (Progenesis® QI)	Mean TIC	CV (%) (technical, *n*=5)	CV (%) (intra-day, *n*=10)
3-Hydroxybutyric acid [Hydroxy acids and derivates	-0.5	103.043	103.042	29.11	19.41	349,971.37	6.82	14.26
Linoleic acid [Fatty acyls]	6.8	279.240	279.243	25.07	35.81	25,346.48	36.88	17.94
L-carnosine [Peptidomimetics]	-4.0	225.104	225.108	17.77	35.54	9,317,926.90	10.02	15.40
DOPG [Glycerophospho lipids]	12.5	773.567	773.574	42.66	51.71	5,370,661.40	15.83	23.41
POPG [Glycerophospho lipids]	12.3	747.549	747.556	41.47	50.83	5,610,977.60	13.59	16.68
D-mannitol [Organooxygen compounds]	-3.1	181.076	181.077	22.09	27.61	1,656,544.50	9.00	10.42
Myo-inositol [Organooxygen compounds]	-3.7	179.061	179.061	27.92	27.92	10,773.33	14.69	20.97
L-cysteine [Carboxylic acids and derivates]	-2.5	120.017	120.015	33.33	16.66	6,996,203.60	13.91	11.40
L-arginine [Amino acids]	-4.2	173.110	173.110	28.88	28.88	82,676.32	5.18	8.44
L-kynurenine [Organic oxygen compounds]	-2.2	207.087	207.084	43.46	28.97	906,788.13	7.37	15.83
Taurolitocholate-3-sulfate [Steroids and derivates]	4.5	562.272	562.272	35.57	35.57	1,482,933.40	14.89	20.96

Table S8.

Analytical standards spiked onto the rectal MetaSAMP® membrane in solvent.

The standards were evaluated for their detectability in negative ionization mode and their measurement characteristics.

Standard ([M-H]$^-$ adduct)	Measured *m/z* (Masslynx™)	Measured *m/z* (Progenesis® QI)	Mass accuracy (ppm) (Masslynx™)	Mass accuracy (ppm) (Progenesis® QI)	Mean TIC	CV (%) (technical, *n*=5)	CV (%) (intra-day, *n*=10)
3-Hydroxybutyric acid	103.042	103.042	19.41	19.41	1,886,469.20	21.15	15.33
Linoleic acid	279.244	279.241	39.39	28.65	73,128.44	27.55	37.95
L-carnosine	225.108	225.103	35.54	13.33	18,667,222.36	20.46	12.49
DOPG	773.457	773.451	99.55	107.31	6,224,078.63	28.89	41.33
POPG	747.608	747.541	120.38	30.77	370,721.73	26.78	39.21
D-mannitol	181.055	181.055	93.89	92.89	2,652,254.25	32.15	11.97
Myo-inositol	179.078	179.039	122.85	94.95	Adduct not consistently detected	NA	NA
L-cysteine	119.997	119.998	133.34	125.00	Adduct not consistently detected	NA	NA
L-arginine	173.086	173.086	109.77	109.77	11,809,530.79	17.96	10.00
L-Kynurenine	207.108	207.048	144.85	144.83	8,627,131.76	9.20	17.64
Taurolitocholate-3-sulfate	562.243	562.263	16.01	19.56	391,494.01	35.85	51.65

Table S9.

Analytical standards spiked directly onto the impregnated rectal MetaSAMP®.

The standards were evaluated for their detectability in negative ionization mode and their measurement characteristics.

Standard ([M-H]⁻ adduct) [class]	Measured *m/z*	Mass accuracy (ppm)	CV (%) (technical, *n*=5)	CV (%) (intraday, *n*=10)	CV (%) (interday, *n*=10 every day (2 days))
3-Hydroxybutyric acid	103.041	9.70	10.15	11.01	18.79
Linoleic acid	279.242	32.23	21.17	26.75	33.50
L-carnosine	225.105	22.21	10.23	14.79	19.11
DOPG	773.555	27.15	15.53	17.81	25.68
POPG	747.538	26.75	13.03	17.00	25.92
D-mannitol	181.076	22.09	4.83	13.57	23.67
Myo-inositol	179.059	16.75	3.59	8.48	16.97
L-cysteine	Adduct not consistently detected	NA	NA	NA	NA
L-arginine	173.100	28.89	8.42	14.50	25.73
L-kynurenine	207.081	14.87	8.25	13.20	89.16
Taurolitocholate-3-sulfate	Adduct not consistently detected	NA	NA	NA	NA

Table S10.

Recovery assessed in the rectal MetaSAMP® *vs.* crude feces using UHPLC-HRMS analysis.

Recovery (%) was evaluated for 16 metabolites by dividing their integrated peak areas obtained following UHPLC-HRMS analysis of rectal MetaSAMP® with those detected in feces (n=5), each normalized by their corresponding specific matched internal standard (in terms of m/z and polarity).

Metabolite (Internal standard)	Recovery (%)
3-Hydroxybutyric acid (Deoxycholic acid-d4)	89.20
Linoleic acid (Deoxycholic acid-d4)	119.14
L-carnosine (Dopamine-d4)	150.70
D-mannitol (L-Valine-d8)	102.31
L-arginine (L-Tyrosine-d2)	108.74
L-kynurenine (Indole-3-acetic acid-d5)	105.29
L-alanine (L-alanine-d3)	98.14
L-valine (Valine-d8)	113.99
Dopamine (Dopamine-d4)	82.07
O-succinyl-L-carnitine (Indole-3-acetic acid-d5)	109.97
N-acetyl-L-glutamic acid (Phenylalanine-d2)	102.37
L-tyrosine (Tyrosine-d2)	100.05
L-phenylalanine (Phenylalanine-d2)	99.59
Isovaleryl-L-carnitine (Indole-3-acetic acid-d5)	109.42
Indole-3-acetic acid (Indole-3-acetic acid-d5)	103.47
Deoxycholic acid (Deoxycholic acid-d4)	112.18

Table S11.

Untargeted and targeted linearity of fecal dilution series using rectal MetaSAMP®-LA-REIMS.

The linearity of untargeted fingerprinting was evaluated for both the rectal MetaSAMP® and feces (*n*=5, 8-point dilution series). The % indicates the number of molecular features that could be detected using specific determination coefficient thresholds for the dilution series tested, relative to the total feature count (*n* =1,222 and 1,183 for rectal MetaSAMP® and feces, respectively). The targeted linearity (0, 33, 50, and 100 ng μL^{-1}) was assessed for the rectal MetaSAMP® on a selection of metabolites (based on tables S7-S9).

	Untargeted % (absolute feature count)	
R^2 threshold	**Rectal MetaSAMP®**	**Feces**
≥0.9	19.9 (243)	18.9 (224)
≥0.8	45.8 (560)	32.5 (384)
≥0.7	60.6 (740)	46.0 (544)
Targeted		
Metabolite	**R^2**	
3-Hydroxybutyric acid	0.97	
Linoleic acid	0.99	
L-carnosine	0.90	
D-mannitol	0.76	
L-arginine	0.83	
O-succinyl-L-carnitine	0.88	
7-Ketocholesterol	0.75	
12-Tridecenoic acid	0.99	

Table S12.

Comparing LA-REIMS fingerprints of MetaSAMP®s impregnated with the different biofluids and their crude counterparts.

Multivariate statistical validation parameters of OPLS-DA models for the LA-REIMS analysis of the MetaSAMP®s and crude biofluids. See Fig. 4. for more details.

Matrix	R^2(cum)	Q^2(cum)	CV-ANOVA *p*-value	VIP≥1	Molecular feature count
Fecal water	0.74	0.40	$4.14\ e^{-6}$	157	1,965
Rectal MetaSAMP®	0.96	0.63	$1.15\ e^{-13}$	827	2,484
Urine	0.73	0.70	$4.97\ e^{-23}$	1,001	2,827
Urinary MetaSAMP®	0.64	0.58	$1.68\ e^{-11}$	1,020	2,848
Saliva	0.74	0.58	$4.06\ e^{-10}$	417	4,993
Salivary MetaSAMP®	0.98	0.53	$4.05\ e^{-5}$	659	5,146

Table S13.

Evaluation of different fecal sample preparations.

The repeatability values were calculated based on TIC-corrected sample intensities.

Fecal sample type	CV (%, *n*=10)	Average % of molecular features with CV ≤30%
Slurry	26.76	0.17
1:2	13.00	66.13
1:3	16.71	34.24
1:4	2.31	83.22
Lipid extract	37.44	58.01
Polar extract	17.74	57.16

Table S14.

Data analysis pipeline.

A comprehensive list of the packages used in the current project accompanied with the version number and implementation module.

Language	Package	Version	Module
R	base	3.4.3	All modules
R	boot	1.3-20	Multivariate analysis
R	car	2.1-3	Visualization of graphics
R	data.table	1.12.2	Univariate analysis; Targeted analysis REIMS
R	datasets	3.4.3	All modules
R	dunn.test	1.3.5	Univariate analysis
R	ggplot2	3.2.1	Visualization of graphics
R	ggbeeswarm	0.6.0	Visualization of graphics
R	graphics	3.4.3	All modules
R	grDevices	3.4.3	All modules
R	methods	3.4.3	All modules
R	moments	0.13	Visualization of graphics
R	NormalizeMets	0.25	Multivariate analysis
R	pcaMethods	1.76.0	Validation statistics; Multivariate analysis
R	RColorBrewer	1.1-2	Visualization of graphics
R	reshape	0.8.7	Univariate analysis; Targeted analysis REIMS
R	ropls	1.10.0	Multivariate analysis
R	stats	3.4.3	All modules
R	stringr	1.4.0	Univariate analysis; Targeted analysis REIMS
R	utils	3.4.3	All modules
Python	dataframe_image	0.1.1	Visualization
Python	FPDF	1.7.1	Visualization
Python	matplotlib	3.3.2	Visualization
Python	numpy	1.19.4	Machine learning
Python	pandas	1.4.3	Machine learning
Python	pyPDF2	2.4.2	Visualization
Python	sklearn	0.23.2	Machine learning
Python	sklego	0.6.12	Machine learning
Python	time	3.7.4	All modules

Data S1.
The feature ions detected in crude feces and impregnated rectal MetaSAMP®s.
(Excel)

Data S2.
The feature ions detected in crude saliva and impregnated salivary MetaSAMP®s.
(Excel)

Data S3.
The feature ions detected in crude urine and impregnated urinary MetaSAMP®s.
(Excel)

Data S4.
The averaged feature intensities detected in 0.1 Da bin sizes and TIC measurement following LA-REIMS analysis of crude feces and impregnated rectal MetaSAMP®s.
(Excel)

Data S5.
The averaged feature intensities detected in 0.1 Da bin sizes and TIC measurement following LA-REIMS analysis of crude saliva and impregnated salivary MetaSAMP®.
(Excel)

Data S6.
The averaged feature intensities detected in 0.1 Da bin sizes and TIC measurement following LA-REIMS analysis of crude urine and impregnated urinary MetaSAMP®.
(Excel)

Data S7.
Standard database.
In-house analytical standard database for metabolomics and lipidomics analysis.
(Excel)

Data S8.
Targeted MetaSAMP®-LA-REIMS analysis.
Visualization of graphs of the metabolite adducts that were detected in negative ionization mode during the targeted analysis of spiked analytes in a solvent (left plot), spiked analytes onto the rectal MetaSAMP® membrane in a solvent (middle plot), and spiked analytes onto the

impregnated rectal MetaSAMP® membrane (right plot) for each component, respectively. If the adduct detected was not reliable then the criteria for disregarding the metabolite were mentioned. The y-axis of the graphs shows the observed intensity for every adduct that was detected in replicated burns. The vertical line displayed in each graph represented the theoretical *m/z* value of the adduct and the window displayed by the x-axis represented a mass window of 150 ppm to enable a reliable detection range.
(Excel)