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A new oil/membrane approach for integrated sweat sampling and sensing: sample volumes reduced from μL's to nL's and reduction of analyte contamination from skin†

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Wearable sweat biosensing technology has dominantly relied on techniques which place planar-sensors or fluid-capture materials directly onto the skin surface. This 'on-skin' approach can result in sample volumes in the μL regime, due to the roughness of skin and/or due to the presence of hair. Not only does this increase the required sampling time to 10's of minutes or more, but it also increases the time that sweat spends on skin and therefore increases the amount of analyte contamination coming from the skin surface. Reported here is a first demonstration of a new paradigm in sweat sampling and sensing, where sample volumes are reduced from the μL's to nL's regime, and where analyte contamination from skin is reduced or even eliminated. A micro-porous membrane is constructed such that it is porous to sweat only. To complete a working device, first placed onto skin is a cosmetic-grade oil, secondly this membrane, and thirdly the sensors. As a result, spreading of sweat is isolated to only regions above the sweat glands before it reaches the sensors. Best case sampling intervals are on the order of several minutes, and the majority of hydrophilic (low oil solubility) contaminants from the skin surface are blocked. *In vitro* validation of this new approach is performed with an improved artificial skin including human hair. *In vivo* tests show strikingly consistent results, and reveal that the oil/membrane is robust enough to even allow horizontal sliding of a sensor.

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Introduction

Recently, it has become clear that eccrine sweat provides a potentially attractive source of ion, molecule, and protein analytes.[1,2] Consequently, there has been a significant increase in technology[3–8] to detect sweat analytes as they emerge onto skin. These new 'wearable' approaches place a planar sensor or sweat-collecting material directly on skin. Placing sensors onto skin quickly resolves[3] the historical challenges in sweat biosensing, such as very large sample volumes, evaporation, lack of sampling devices, and need for a trained staff.[9] However, the current focus of most researchers[1–8] is primarily for *continuous* monitoring, not for one time sampling which is arguably already well-served by drawing blood. This creates challenges beyond those encountered historically for sweat,[3] especially because current approaches in placing sensors or sampling materials against skin limit sampling rates to 10's of minutes or even 100's of minutes for lower sweat rates.[1,3] We are not aware of any reports which address this critical challenge with sample volumes and sampling rates. Slow sampling rates also mean that sweat spends more time sitting on the skin surface, which will only increase analyte contamination coming from the skin surface.[10] Reducing skin contamination has been achieved for clinical (one-time) sampling,[10] but is a clear challenge that remains unresolved for wearable and continuous sensing of analytes in sweat.[3]

Reported here is demonstration of a novel oil/membrane approach for sweat sampling and sensing. This report represents the beginning of a new paradigm where sample volumes are reduced from the μL's to nL's regime, and where analyte contamination from skin is reduced or even

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eliminated. A micro-porous membrane is coated with a dissolvable polymer such that it is porous to sweat only, and then is coated with a cosmetic-grade oil and placed onto the skin (Fig. 1). As a result, sweat is then isolated to regions only above the sweat glands before it reaches the sensors. This greatly reduces the sample volume and therefore the sampling rate is proportionally increased. Best case sampling intervals are on the order of several minutes, and the majority of hydrophilic (low oil solubility) contaminants from the skin surface are blocked. Validation includes *in vitro* demonstration with and without human hair. As part of the *in vitro* validation, we also present an improved artificial microfluidic skin which is simpler to fabricate and operate than our previously reported approach.[11]

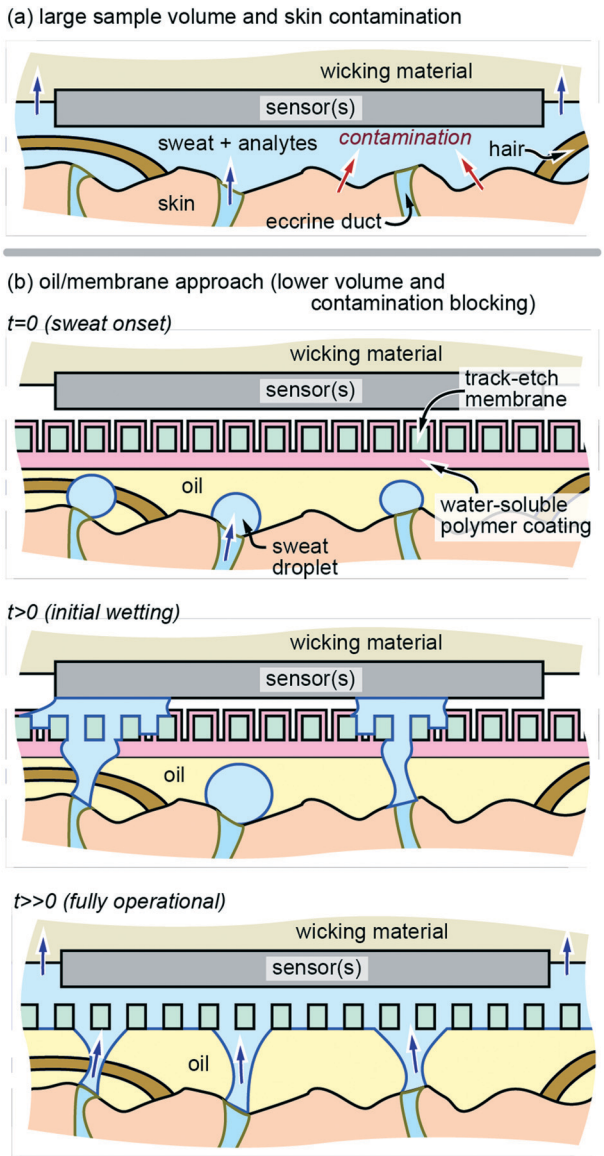


Fig. 1 Diagrams of sweat sensing on skin with (a) the conventional approach,[1–8] and (b) time-lapse diagrams of the oil/membrane approach of this paper which reduces volume and skin contamination.

In vivo validation is also performed with an electrical-impedance sensor, revealing that the technique is robust enough to even allow sliding of a sensor over the membrane/oil. The results reported here may represent the beginning of a new regime for sweat biosensing, where even smaller amounts of sweat are sampled due to more sophisticated interfacing of technology on skin.

Background and design

There are two goals that motivated pursuit of this work. The first was to reduce the required sweat volume (*e.g.* the total volume that must be refilled and replenished between sensors and the eccrine ducts).[1,3] The second, was to isolate eccrine ducts from the skin surface to reduce possible contamination by analytes coming from the skin surface, from dead cells, or from microbes.[3,10] Both of these goals become most important for low or intermittent sweat generation rates (increased time for contamination). At very high sweat rates (nL's min^{-1} per gland), the value of the oil/membrane approach reported here is still beneficial but reduced. Practically, continuously obtaining high sweat rates is likely limited to applications like athletics, or will require chemical stimulation.[3]

We are not aware of any published results in the first goal of reducing the required sweat volume. However, the use of petroleum jelly or oil is well known for imaging sweat production[12] as shown in Fig. 2b. Furthermore, placing oil on skin has also in one instance been used clinically by Boysen *et al.* (no sensors, bag collection of sweat) to reduce analyte contamination from the skin surface.[10] Boysen's work clearly reveals both the potential advantages and limitations of the work presented here. As shown in the summary of Table 1, *even after pre-washing and rinsing of the skin*, without a layer of oil there is significant contamination for hydrophilic and large analytes. Boysen's work concluded that the contamination was due to elution of water-soluble contaminants into sweat from the epidermis. However, for small lipophilic analytes (*e.g.* cholesterol) which are highly soluble in oil, the addition of a layer of oil had no effect on blocking

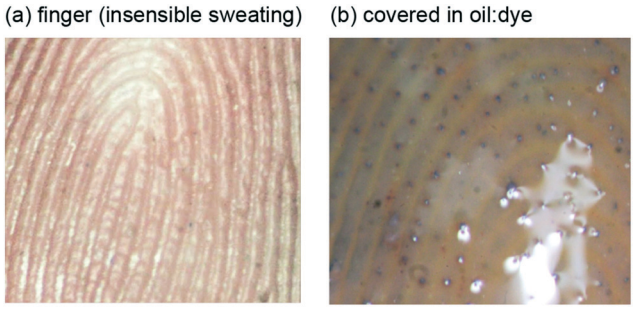


Fig. 2 Use of oil with bromophenol blue pH dye on skin for imaging sweat emerging from ducts (b) even at sweat low sweat generation rates that otherwise are (a) invisible and insensible due to evaporation.[12]

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Table 1 Evidence of contamination in initial sweat samples collected into a bag with: dripping sweat collection with an oil layer on skin (baseline); dripping sweat collection without an oil layer on skin; scraping sweat collection without an oil layer.[10] cAMP is cyclic adenosine monophosphate. Skin was washed/rinsed/dried before collection

Analyte	M.W. (Da)	Drip collect & oil (baseline)	Drip collect & no oil	Scraping & no oil
Calcium	40	~0.25 mM	+150%	+500%
Urea	60	~4 mM	+40%	+150%
cAMP	329	~0.2 nM	+200%	+650%
Protein	10's k	~25 mg dL^{-1}	+60%	+150%
Cholesterol	387	~0.25 μM	No change	+450%

contamination from the skin surface. Therefore, the oil/membrane approach is unlikely to provide significant advantages for small lipophilic analytes such as hormones (*e.g.* cortisol).

With additional reference to the 'scraping' column in Table 1, it should be noted Boysen's work further suggests that sweat biosensing approaches should not utilize sampling materials (*e.g.* textiles) or sensors that repeatedly slide across the bare skin (no oil or skin sealant). Such approaches will likely increase the contamination from skin and should be avoided for high-quality biosensing. Lastly, we note that although Boysen showed that at high sweat rates eluted contamination is mitigated over time, at low or intermittent sweat rates significant contamination is likely to occur.

With a greater appreciation now understood on the value of a layer of oil for sweat collection, the discussion now turns to the device design. Foremost, use of an oil layer is not readily translatable to microfluidic sampling or sensors on skin because the oil would likely foul the sensor surface and/or clog the sampling materials. To solve this problem, the novel oil/membrane approach reported here (Fig. 1b), places a membrane in between the oil and the sensors, and the membrane is only porous to sweat. A conventional membrane is unlikely to work, as oil has a lower surface tension than sweat, and would easily wet through any dry membrane, and likely foul the sensors as well. In this work, the membrane is coated with a sweat (water) dissolvable polymer which blocks oil permeation. In all cases, only sweat will wet the pores in the membrane, and because the membrane is hydrophilic, capillary pressure ensures that sweat remains wetted into the pores. Consider for example a track-etch membrane with d = 5 μm diameter pores and a sweat contact angle on the membrane in oil of θ = 45°. Assuming an interfacial tension between the sweat and oil of γ = 50 mN m^{-1}, the resulting capillary pressure is $\Delta p = 2\gamma\cos(\theta)/(d)$ = 14 kN m^{-2} (2 psi). Such pressure is more than adequate to retain separation of the oil from the sensor. Furthermore, track-etch membranes even with <100 nm pores are commercially available. Lastly, we note that another design advantage of adding the membrane is that sensors are moved off the skin which can introduce electrical noise from or through the body. Although not demonstrated in this work, simply providing a hydrophilic gold or other type of electrode coating on the membrane and electrically grounding this electrode coating, would block this route for entry of electrical noise.

Theoretical calculation of sweat sample volume

Until this report, all previous sweat tattoos,[4,5] patches,[6] bands,[8] or devices[7,13] create a sweat volume between a planar surface pressed on skin and the skin itself.[1] This sweat volume can be problematic if rapid sampling is desired, as will be seen through several simple calculations, and as observable through previous modeling work.[1] Assume a skin roughness peak-to-valley height of ~60 μm (ref. 11 and 14) and a single sensor or array of sensors comprising 0.5 cm^2 (*e.g.* 4 mm radius). The resulting sweat volume between sensor(s) and skin can be estimated to be 1.5 μL, and would be worse in the presence of hair or topographical skin defects. Next, assume 100 active sweat glands per cm^2 (*e.g.* the forearm) at a moderate sweat generation rate of 1 nL min^{-1} per gland.[1] It would require 30 minutes to fill this volume initially, and after filling, to fully flush out all old sweat it could potentially require ~4× longer or 120 minutes.[1] Now, instead assume the oil/membrane device construction in this work (of Fig. 1b). Assume the sweat volume between the membrane and the skin is bounded by oil, to 'spheres' of sweat above each gland with diameter that is on average half the peak-to-valley height of skin (~30 μm, ~14 pL). Using the same sensor area stated previously, the 50 sweat glands would therefore present a total sweat volume in oil of ~0.7 nL (2000× lower than the case without oil). Not to be forgotten, there still exists a volume between the sensor and the membrane. Assuming that volume were 5 μm thick and packed with nanosilica or other material with 40% porosity, the resulting sweat volume is still 15× lower (100 nL) than the case with no oil. With hair or topographical skin defects this improvement could diminish for a spherical sweat droplet assumption, because the total volume (oil and sweat) is only proportional to the gap between sensors and skin, whereas the volume of spheres of sweat scales as the gap cubed. However, with a strongly wicking material (depicted in Fig. 1) such spheres would collapse as soon as they touch the membrane, reducing the sweat volume.

Based on the above estimates for reduced sweat volume, the sweat sampling times can be reduced from the 30–120 minutes mentioned above, to 2–8 minutes. Alternately, lower sweat volume can allow of a greater number of viable natural sampling events (*e.g.* a device could work at lower sweat generation rates). Furthermore, if chemical sweat stimulation is used[3] then allowing lower sweat generation rates reduces the total amount of stimulant needed.

Membrane fabrication and oil selection

In this section we describe the fabrication steps for the oil/membrane used for both *in vitro* and for *in vivo* validation.

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Hydrophilic (PVP coated) polycarbonate track-etch membranes were purchased from SPI Supplies with a thickness of ~10 μm, pore diameter of 5 μm and pore density of ~400 000 per cm^2 (~8% porous area). A ~2 μm thick polyvinyl alcohol (PVA) film was prepared on a glass or plastic sheet by spin or dipcoating an aqueous PVA solution, followed by air-drying for 2 min. Air-drying of the PVA was chosen to provide incomplete drying and improved tackiness for lamination to the track-etch membrane. The partially air-dried 2 μm PVA film was then transferred to the track-etch membrane by lamination using a Western Magnum XRL-18 laminator at room temperature with 5 psi (see resulting images in Fig. 3). To ensure rapid solubility by sweat PVA was specifically chosen which was 80% hydrolyzed and with 9–10 kDa molecular weight. At first, it was speculated that a micro-replicated jagged surface texture may have been needed on the lower surface of the PVA film to ensure more rapid wetting by sweat in oil, but in experiments, a planar PVA surface was found to be fully adequate, resulting in wetting and dissolution of the PVA in <10 s.

For the oil, a 1 : 2 blend of cosmetic-grade (skin-compatible) ClearCo PSF-5000cSt oil and ClearCo PSF-100cSt oil was chosen. This dimethicone oil blend has a surface tension of ~21 mN m^{-1}. In early *in vitro* testing work, ~1 wt% of polyglucose cosmetic-grade surfactant was added to the oil to promote more rapid wetting of the sweat to the PVA surface (the surfactant is only sweat soluble, and was solubilized into the oil with alcohol and sonication, followed by evaporation of the alcohol). However, in the experiments reported here, it was found that wetting was adequately fast even without surfactant (*e.g.* no surfactant was used in the experimental results shown in the next sections).

Artificial microfluidic skin for *in vitro* testing

For the *in vitro* validation an improved method for fabrication of the artificial microfluidic skin was implemented (Fig. 4). This new method uses more widely available materials, and is simpler to fabricate and integrate than our previously reported approach.[11] The fabrication starts with applying REPLIFLO™ mixed resin (Cuderm Corp.) to human skin and peeling it off to make an inverse mold of skin texture (in this work, the lead author's forearm was used). Then the inverse skin texture is hot-roll-laminated (95 °C, 10 psi) into a 50 μm thick film of Dupont MX5050 dry film negative-photoresist layer. Note, the underlying polyester backing film for the MX5050 is not removed. With the photoresist film imprinted with the skin pattern, the photoresist is cured with UV light (365 nm, 4 mW cm^{-2}, 45 s). Next a thinner 15 μm thick DuPont MX5015 photoresist film is laminated to the polyester backing of the microreplicated MX5050 film. This MX5015 is simply added to act as a dry-adhesive. This film stack is laser micro-machined with one or more 'eccrine sweat pores' using an Universal Laser Systems VLS3.50 system. Lastly, a track-etched polycarbonate membrane (hydrophilic, 10 μm thick, pore diameter ~5 μm, Whatman) is laminated onto the bottom adhesive MX5015 layer. The completed artificial microfluidic skin membrane was then epoxied onto a 13 mm polycarbonate membrane filter holder (SPI Supplies #F0101-BA), which allowed easy connect/disconnect with the tubing coming from the syringe pump (NE-1000, New Era Pump System, Inc.). To simplify data collection, a single and centered artificial pore was utilized.

The fully fabricated module is visible in the inset photo of Fig. 4b and c. The large black circular region in Fig. 4c is

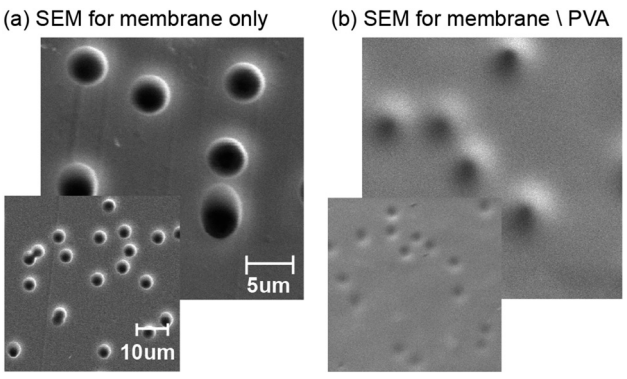


Fig. 3 Scanning electron microscope (SEM) images of a single membrane: (a) top-side (uncoated) and (b) bottom-side (PVA-coated).

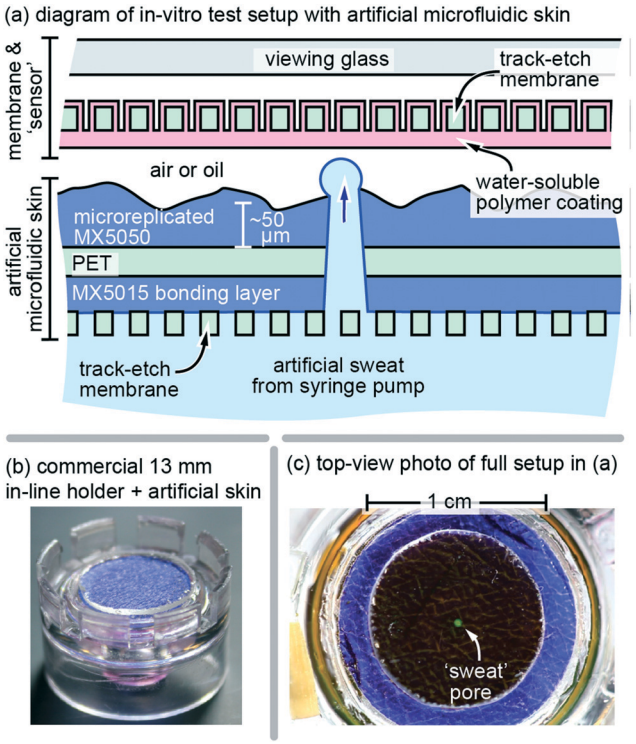


Fig. 4 *In vitro* testing setup with (a) diagram of the artificial microfluidic skin (improved from our previous work[11]) and inset photos of (b) the completed module and (c) the completed module as it starts to fill with artificial sweat.

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simply because the photo is of the module at the beginning of use, and the semi-transparent artificial skin appears darker where the yellow-tinted artificial sweat is wetted behind it (water + 0.5% disodium fluorescene dye). This backside area wetted by artificial sweat is ~1 cm in diameter (~0.8 cm^2).

In vitro validation

In vitro validation of the oil/membrane approach was explored using the testing setup shown in Fig. 4. A glass disk was utilized in place of an actual sensor to allow imaging of the horizontal spreading of the artificial 'sweat', which in all cases was water with 0.5% disodium fluorescene fluorescent dye. For these experiments, the syringe pump was set to 500 nL min^{-1}. For the total area (~0.8 cm^2) of the artificial skin, on the forearm where the active gland density would be ~100 glands per cm^2, the single centered pore at 500 nL min^{-1} per gland translates to 80 actively firing glands with individual sweat generation rates of 6.25 nL min^{-1} per gland. 6.25 nL min^{-1} per gland is near the upper natural sweat generation limit of 5–10 nL min^{-1} per gland.[1]

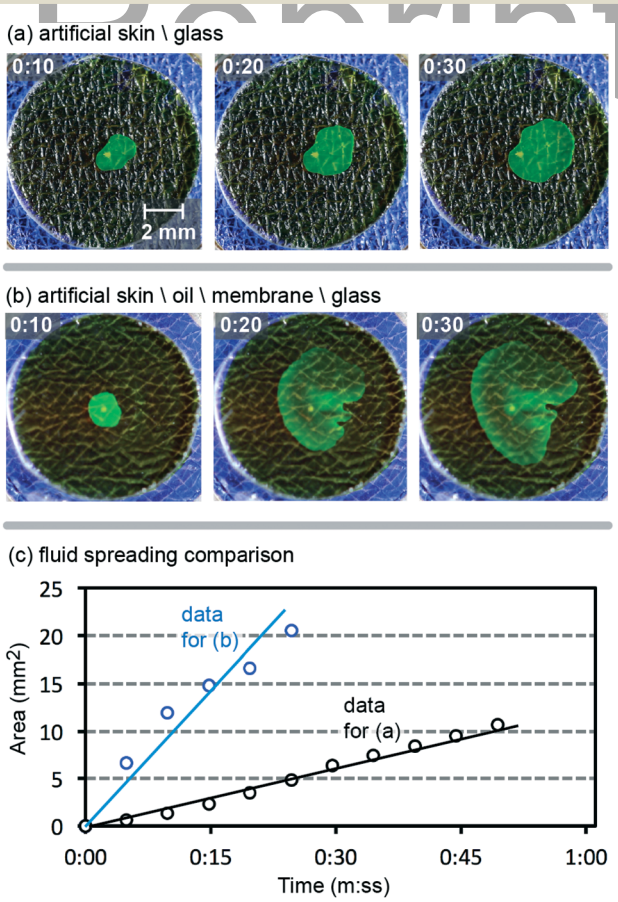


Fig. 5 *In vitro* testing results including (a and b) time-lapse photographs of fluid spreading with and without inclusion of oil in the device of Fig. 4, and (c) plot of fluid area *vs.* time as measured by ImageJ, further confirming reduced sweat volume.

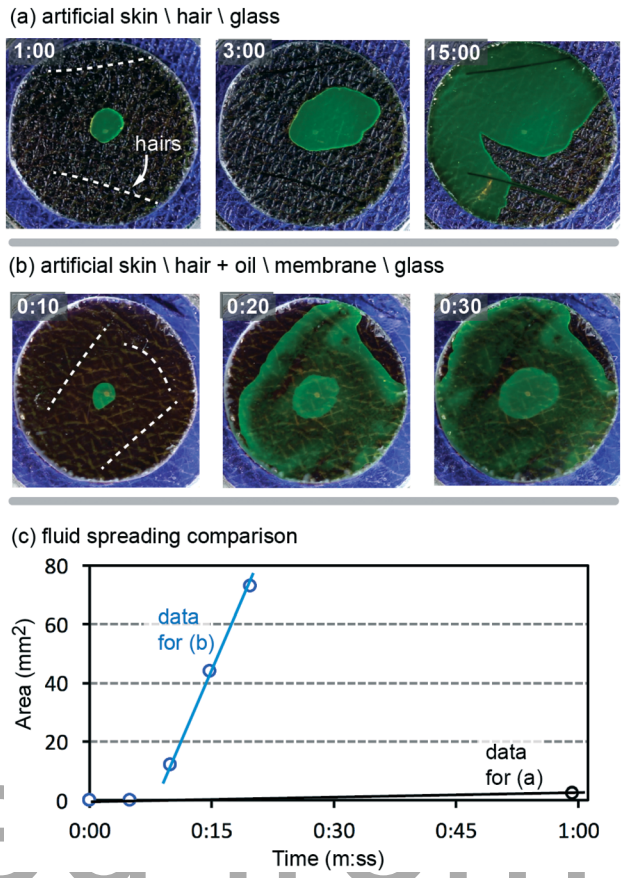


Fig. 6 *In vitro* testing results ***with hair from the lead author***, including (a and b) time-lapse photographs of fluid spreading with and without inclusion of oil in the device of Fig. 4, and (c) plot of fluid area *vs.* time as measured by ImageJ, further confirming reduced sweat volume.

As seen visually in experimental photos of Fig. 5 and 6, a central bright spot reveals the location of the artificial sweat pore (even in spreading photos the location of the pore is brighter, because the fluorescent 'sweat' is thicker there). In Fig. 5a, the glass slide is placed directly onto the artificial skin, and the increase in spread area of the 'sweat' occurs relatively slowly due to larger volume between the glass slide and the artificial skin. For this case with no oil, after 30 s the incoming flow should total approximately 250 nL, and the ~6 mm^2 spread area suggests that the average gap between the glass and the artificial skin is ~42 μm (a realistically expected value for real skin as well). In Fig. 5b, both the oil and membrane are added, resulting in a faster spreading rate, and a visibly dimmer fluorescence from the 'sweat'. Both faster spreading with the addition of oil (Fig. 5c) and dimmer fluorescence (Fig. 5b) indicate that the 'sweat' is only filling the smaller volume between the glass slide and the membrane (*i.e.* the oil is functioning as expected). In all experiments, the wetting and dissolution of the ~2 μm PVA film appeared to be rapid (<10 s). Again, using flow-rate/volume estimates, the average gap between the glass and membrane can be calculated to be ~8 μm. Therefore, the oil/membrane approach, in this single *in vitro* example, improved the

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sampling rate by at least 5×. Exact quantitative results are not the most important aspect of Fig. 5, rather, more importantly, Fig. 5 validates that the oil/membrane approach works as diagrammed in Fig. 1.

Some further data interpretation is provided. The experimental data in Fig. 5 predicts that the sampling interval based on simply refilling the sweat volume[1] would be ~6 minutes without oil and ~1 minute with oil. Again, this is for a average sweat generation rate of ~6.25 nL min^{-1} per gland, and natural sweat rates would often be ~10× lower (*e.g.* ~0.6 nL min^{-1} per gland would result in 60 minutes/10 minutes). Further consider that for multiple sweat ducts, the worst case sampling rate could be 4× slower to fully displace all the old sweat with new sweat[1] (240 minutes/40 minutes). This clearly demonstrates the need for reduced sweat volume.

A test similar to that of Fig. 5 was repeated in Fig. 6, but with the addition of human hairs from lead author (average hair diameter of ~100 μm). With hair and no oil/membrane, the spreading is extremely slow (note the time scale in Fig. 6a is up to 15 min). The case photographed in Fig. 6b where the oil/membrane is added results in dramatically faster spreading of the 'sweat'. The results of Fig. 6 for oil/membrane are even faster than that of Fig. 5, suggesting a smaller gap between the membrane and cover glass. The increase in spread area was also observed to be non-linear, which is expected as a variation in gap height between the glass and membrane is visually apparent based on fluorescent brightness of the artificial sweat. Also noted for Fig. 6, after 20 s the 'sweat' starts to fill the lower channel between the membrane and skin. Compared to Fig. 5, this is likely due to a larger gap height between the membrane and skin, which causes a lower Laplace pressure for the 'sweat'. In practice, with a wicking material above the sensors (Fig. 1) and with more typical (lower) sweat generation rates one would not expect such spreading of 'sweat' between the membrane and skin. In fact, if the wicking pressure beyond the membrane is strong enough, a capillary bridge between the membrane and sweat ducts could be repeatedly broken and reformed during use of the device.

The results of Fig. 6 also reveal a significant advantage of our oil/membrane approach: no skin prep other than application of oil is required (no washing, no hair removal, *etc.*). In practice, application of the device might be quite simple, especially if a higher viscosity petroleum gel or oil is pre-applied to the membrane surface, allowing a one-step application of the device onto skin. It is important to note that the results of Fig. 5 and 6 will vary from test to test, as will also be the case for real-world testing. The reader is therefore reminded again, that the most important result of Fig. 5 and 6, is to reveal that the oil/membrane approach is working as expected and as illustrated in Fig. 1. Lastly, the reader is also reminded that in real practice, the membrane might be applied to the sensor in a manner where the separation between the membrane and sensor can be predictably regulated (*e.g.* rigid spacers, or the space between the membrane and sensor can be filled with an agar film).

In vivo validation – rationale for using an electrical impedance sensor

In vivo validation of the oil/membrane approach was performed using skin-impedance measurement, a method that will closely track the amount of sweat penetration occurring through the membrane. Skin-impedance measurement, also known as galvanic-skin-response,[1] is a highly sensitive method for indirectly measuring changes in sweat rate. The 10–20 μm stratum corneum of the skin has a very high electrical impedance. A low-impedance route into the skin is available through hair-follicles and sweat ducts, and this impedance is even lower when the sweat ducts are filled with salty sweat.[1] As will be seen, skin impedance is further useful for *in vivo* validation of the oil/membrane approach for two reasons: (1) impedance allows quantified verification of the presence of each layer of material used in the oil/membrane approach (Fig. 1), as the oil/membrane without sweat should have a very large electrical impedance; (2), any broken connection between the sweat ducts and the sensor can be detected by an increase impedance (an analyte sensor would not show this, because it could remain wetted with sweat and analytes even if this connection were broken). For both of these reasons, we chose skin-impedance for validation over an electrochemical analyte sensor (reserved for future work).

One additional benefit of the oil/membrane is that for skin impedance measurements, it can isolate impedance to primarily that of the ducts only. Therefore, if one wishes to closely explore the impedance of sweat ducts during sweating, this oil/membrane approach would eliminate possible confounding impedance from the stratum corneum, lesions or defects, and/or hair follicles. Ultimately this could lead to a device with more accurate sweat generation rate (nL min^{-1} per gland) measurement by impedance, especially so if sodium or chloride concentrations are also measured.[1]

In vivo validation – experiment and results

For *in vivo* validation of the oil/membrane approach by impedance, gold-coated stainless-steel studs (8.2 mm diameter or 0.53 cm^2 each) were band/velcro strapped to the test site using a laser machined acrylic piece shown in the inset photos of Fig. 7. These electrodes were then connected to a Gamry Potentiostat system which provided an impedance-measuring sinusoidal waveform of 2 kHz and 1 V peak-to-peak with data logged every three seconds. The test site, as shown the inset photo of Fig. 7b, and as can be seen includes hair. The test site was 1.9 cm^2 which matches the area of the carbachol gel disk used for iontophoretic sweat stimulation. The impedance electrodes were placed such that one of the electrodes was always on clean and non-sweating skin, whereas the other electrode was on the site where oil/membrane and stimulation actions were applied. Each time the skin was

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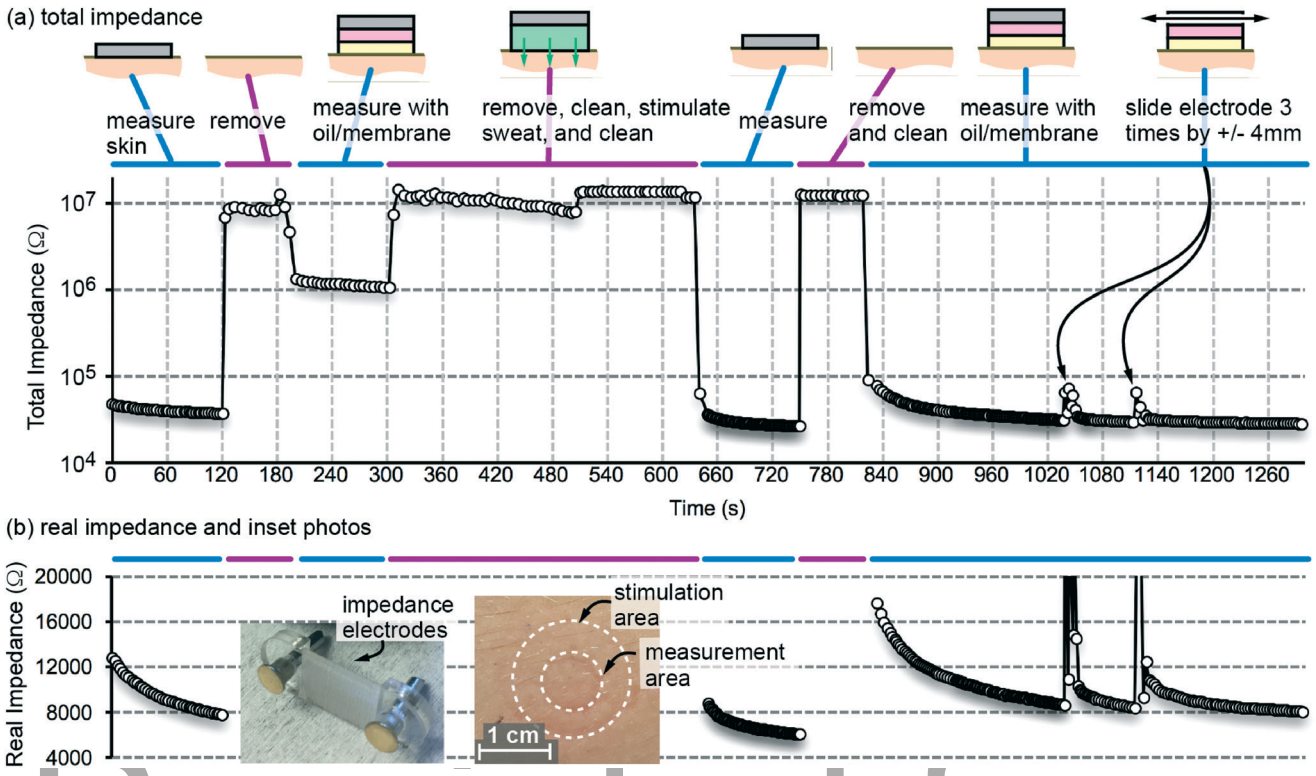


Fig. 7 *In vivo* testing results including (a) total impedance *vs.* time and (b) real impedance *vs.* time. Actions taken are marked with colored segments above each plot with descriptions of actions above the segments in (a). Total impedance includes imaginary (capacitance) and real (resistance), and is used in (a) because it can provide measurement which reveals the presence of the membrane and oil without sweat (*i.e.* very large resistance and capacitance dominant). Real impedance is plotted in (b) which directly measures the conduction through the eccrine ducts. Inset photos of the test apparatus and of the test site is provided in (b). The *in vivo* validation of this figure was repeated several times (see online ESI†), with strikingly similar results each time.

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wiped clean, it was wiped in the order of: 3× isopropyl alcohol wipe/3× water wipe/3× dry wipe. In the data, which will be discussed next, impedance generally decreases with time (except for open-circuit impedance). Several possible causes are given in advance to aid understanding of the data. Gradual decreases in impedance generally indicate: (1) mild electroporation of the skin and tissue around the eccrine glands;[15] (2) hydrating, softening, and flattening of the stratum corneum roughness (more and/or closer electrode contact); (3) for data near the end of the experiment, increased sweat permeation and electrical conduction through the track-etch membrane. Because the voltage used for impedance measurement is so low, electroporation is not likely a contributing factor to gradual impedance reduction.

As shown in the logarithmic plot of Fig. 7a, after a first wipe cleaning of the skin the baseline skin impedance was total ~40 kΩ in Fig. 7a and real ~10 kΩ in Fig. 7b. Near 120 s the impedance electrodes were then removed and open-circuit total impedance (~10 MΩ, Fig. 7a) occurred while the oil and membrane were applied to the test site. No data is shown in several instances of the real impedance (resistance) data of Fig. 7b because in these instances the real impedance is distinctly greater in magnitude. These instances include no electrode contact and oil/membrane placement when there is no sweating. Next, at ~200 s, with the oil/membrane and electrodes in place, a total impedance of ~1 MΩ indicated the presence of the oil/membrane without sweating (electrical capacitance dominant). Next, near 300 s, the electrodes and membrane were removed, and the site wiped clean of the oil, followed by sweat stimulation with carbachol iontophoresis. Iontophoresis of carbachol was performed for 2.5 minutes at 0.5 mA – comparable to values by the commercial Nanoduct product from Wescor. Carbachol was chosen because it is slowly metabolized[3] and we have observed that a single stimulation can last greater than 24 to 48 hours. After the stimulation and another wipe cleaning were completed, at ~640 s the impedance of skin was again measured and found to be on the order of 30 kΩ total, around 7 kΩ real, and rapidly decreasing (increasing sweating). Even though one of the electrode leads was on the sweating skin site (lower impedance), most of the real impedance is likely that of the counter electrode on dry skin (the counter electrode was not on a sweat stimulated site). At ~750 s, the electrodes were again removed, and the testing site quickly wiped dry of sweat, and a new layer of oil/membrane applied. At ~820 s, the electrodes were again placed on the test site and nearly immediately the impedance dropped to a range which could only be possible with electrode-to-gland

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connection *via* sweat. Therefore, like the *in vitro* testing, it appears that wetting and dissolution of the ~2 μm thick PVA film is very rapid. For the measurements taken while sweating, the oil/membrane may slightly increase the real impedance (the membrane is ~8% porous and the pores have a 2 : 1 aspect ratio).

One final and important test was performed twice near 1040 s and 1110 s. The electrode at the test site was slid horizontally three times by both ±4 mm. The data clearly shows that the electrical conductance rapidly recovers. Such capability is critically important, because in a wearable format a sweat biosensor is easily bumped or will have some sliding on skin as local tissue or muscle is stretched or compressed. This data shows that the oil/membrane approach is also compatible with bumping or movement. This is generally expected, because the oil is given no pathway through the membrane (*e.g.* the membrane is accessed always first by sweat).

Lastly, it is important to note, that the entire experiment of Fig. 7 was repeated for seven subjects in total, including electrode sliding, and in all cases the results are strikingly similar (see online ESI†). No test data was excluded from the results (100% success rate in this limited trial). This shows preliminary promise to the high reliability and reproducibility of this approach.

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General discussion

This paper conclusively shows a new method which may place sweat sampling and sensing in an entirely different paradigm of sample volumes (μLs to nLs) and sample purity (reducing water soluble skin contaminants). Regarding sample volumes, the oil/membrane approach provides a novel solution to the 10's to 100's of minutes sampling rate limitation expected when placing a sensor directly onto skin.[1] Regarding sample purity, prior clinical studies[10] have clearly shown evidence of skin contamination even at very high sweat rates (sauna induced sweating, Table 1), which can be significantly mitigated by the oil/membrane approach presented here. Furthermore, convenient sweat sampling, more sampling events, and safer/longer sweat stimulation, all require accessing sweat not in the 1's of nL min^{-1} per gland range of sweat generation, but rather in the 0.1's of nL min^{-1} per gland range. This highlights the need to reduce the sweat volume, but also increases the need to mitigate analyte contamination from the skin surface. The contamination from skin would undoubtedly be much worse at these lower sweat generation rates, as without the oil/membrane approach, the sweat under a sensor could sit against the skin for an hour or more at such low sweat generation rates. A further advantage of the proposed approach is that it requires no skin prep, such as skin cleaning and/or hair removal. In a wearable format, skin-prep could be as minimal as just washing the surface to aid adhesion of an adhesive wearable patch,[6] for example.

The *in vivo* data (Fig. 7) showed that sliding of the sensor did not prevent regeneration of a pathway between sweat and the sensing electrode. Even if such sliding caused sweat to become trapped in the oil, trapped sweat has only two options, and neither option is expected to be highly detrimental: sweat can remain in the oil, or move through the membrane onto the sensor. Longer term testing (hours, even a day) is certainly needed, but is beyond the scope of this work. We speculate that if challenges occur, the oil, the membrane material, surface texture, and geometries, can all be optimized to ensure that pathways through the membrane remain wetted with those fluids that wet them first (*i.e.* sweat). Such capability may be easily achieved by using pathways with surfaces with very high wetting hysteresis (wetting pinning). In addition, alternate oil rheology can be used (*e.g.* like petroleum jelly). Furthermore, any oil leakage could be potentially mitigated at least in part, by techniques such as placing between the sensor and membrane a 5–10 μm thick layer of agar hydrogel with oleoscopic microbeads (which could also act as a spacer material).

We do not recommend in practice, that repeated sliding of the collection site should be permitted. Repeated sliding would undoubtedly increase contamination as described for Table 1. Ideally, sampling would be performed on a skin area such as the lumbar back or abdomen where skin is not overly flexed, and the sampling area fixed securely to the skin. Because this approach reduces the sample volume, the sample area could be less than 1 cm^2, and the surrounding adhesive area could be an order of magnitude larger to help minimize sliding and/or detachment from the skin.

We fully recognize that future validation is also required with analyte specific sensors (ions, molecules, *etc.*), but we note that such experimentation will likely be very analyte specific. Different analytes will have different oil/sweat solubilities, different sweat rate requirements due to possible dilution,[3] and different sampling time requirements for practical application. Also, different sensors could have different interference from dissolved PVA, however, because of the reduced sample volume provided by this oil/membrane approach, excess PVA would be washed away rather quickly. In addition, one could use alternates to PVA such as sucrose.

Another challenge with the present approach is integration with repeated sweat stimulation (for sedentary subjects). We speculate that sweat stimulants could be dispersed in the oil (much like that done for dye, Fig. 2) and be delivered into the skin by diffusion. But such demonstration remains unproven. Carbachol stimulant was chosen because it is slowly metabolized[3] and we have observed that a single stimulation can last greater than even 24 to 48 hours. Therefore, a long-duration use-scenario could be enabled with a single stimulation. Slowly metabolized stimulants would also be required if stimulation was achieved by diffusion.

Lastly, while we understand that there may be other viable solutions for reducing sweat volume, we are confident that this work represents the type of research that is needed if

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sweat biosensing is to reach its ultimate potential for non-invasive biomarker access and sensing.[3]

IRB protocol and conflicts of interest statement

The *in vivo* study which requires sweat stimulation and oil placement on skin was performed under Univ. of Cincinnati IRB protocol study ID 2015-5211.I. Corresponding author Jason Heikenfeld has an equity interest in Eccrine Systems, Inc., a company that may potentially benefit from the research results, and also serves on the company's Board. The terms of this arrangement have been reviewed and approved by the University of Cincinnati in accordance with its conflict of interest policies.

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