

Bioinspired Microfluidic Wearable Sensors for Continuous Sweat Sampling and Metabolic Monitoring

Thesis by
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ABSTRACT

Wearable sweat sensors have shown strong potential for noninvasive health monitoring by providing access to dynamic biochemical information. However, continuous monitoring of sweat metabolites remains challenging due to low and variable sweat rates, inefficient sampling, and signal distortion arising from evaporation, contamination, and accumulation. These limitations restrict the accuracy and practical deployment of current wearable systems.

In this thesis, we present bioinspired microfluidic strategies to enable continuous sweat sampling, transport, and metabolic analysis under physiologically relevant conditions. We demonstrate how a Janus interface with asymmetric wettability and geometry enables directional vertical transport at the inlet and outlet, while hierarchical microchannel guides flow across the microfluidic and sensing regions. These design elements enable transport even at low sweat rates while preventing stagnation and backflow, resulting in continuous sweat transport without the need for active pumping. This extends the effective sampling window and reduces the number of sweat induction sessions required.

The platform is integrated with electrochemical sensing modules for real-time detection of metabolites and is further extended toward multiplexed sensing of biomarkers relevant to metabolic health. The system is validated through on-body human studies under stimulated conditions, with initial evaluation during exercise-induced sweating.

Overall, the works presented here establish design principles for coupling sweat induction, sampling, and sensing, advancing wearable biofluid technologies for continuous and noninvasive metabolic monitoring.

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NOMENCLATURE

- Ag.** Silver.
- Alc.** Alcohol.
- ATP.** Adenosine triphosphate.
- Au.** gold.
- BMS³.** Bioinspired microfluidic sweat sensor system.
- BSA.** Bovine Serum Albumin.
- C.** Carbon.
- CE.** Counter Electrode.
- CGM.** Continuous glucose monitoring.
- CV.** Cyclic Voltammetry.
- GA.** Glutaraldehyde.
- Glu.** Glucose.
- IP.** Iontophoresis.
- IRB.** Institutional Review Board.
- NAD⁺.** Nicotinamide adenine dinucleotide, oxidized.
- NiHCF.** Nickel hexacyanoferrate.
- PANI.** Polyaniline.
- PB.** Prussian Blue.
- PCB.** Printed Circuit Board.
- PET.** Polyethylene terephthalate.
- PI.** Polyimide.
- RE.** Reference Electrode.
- SEM.** Scanning Electron Microscopy.
- UA.** Uric acid.
- WE.** Working Electrode.
- XA.** Xanthine.

Chapter 1

INTRODUCTION AND MOTIVATION

Materials from this chapter appear in “Min, Jihong, Jiaobing Tu, Changhao Xu, Heather Lukas, Soyoung Shin, Yiran Yang, Samuel A. Solomon, Daniel Mukasa, and Wei Gao (Mar. 2023). ‘Skin-Interfaced Wearable Sweat Sensors for Precision Medicine’. In: *Chemical Reviews* 123, pp. 5049–5138. doi: 10.1021/acs.chemrev.2c00823” and “Shin, Soyoung, and Wei Gao (May 2026). ‘Making Sweat Measurable: Induction, Sampling, and Refreshment in Wearable Biofluid Sensing’. In: *Advanced Science*. doi: 10.1002/advs.75684.” [1, 2]

1.1 Metabolic Syndrome and the Need for Continuous Monitoring

Metabolic syndrome is a complex metabolic disorder associated with an increased risk of cardiovascular disease, diabetes, and stroke. Affecting approximately one in three adults in the United States, it is characterized by a cluster of interrelated conditions, including abdominal obesity, hypertension, hyperglycemia, elevated triglycerides, and reduced HDL cholesterol [3–5]. Given the chronic and progressive nature of these abnormalities, regular monitoring of metabolic biomarkers such as glucose, lactate, triglycerides, and uric acid is essential for effective prevention and management.

Despite the recognized importance of monitoring multiple metabolic biomarkers, several key research gaps remain, particularly in enabling non-invasive approaches for metabolic syndrome management. First, there is a lack of sensing platforms capable of continuous, non-invasive monitoring of multiple metabolic-syndrome-related biomarkers. Most existing home monitoring approaches still rely on invasive blood sampling or disposable strip-and-meter formats and are typically limited to single-analyte measurements [6, 7].

Continuous glucose monitoring (CGM) systems have partially addressed this challenge by enabling real-time glucose tracking and improving diabetes management. However, CGM devices still require minimally invasive sensor insertion, limiting their suitability as truly non-invasive monitoring solutions [8, 9]. Moreover, current CGM technologies are restricted to glucose sensing and do not provide access to the broader range of biomarkers relevant to diabetes progression and metabolic syn-

drome. Given the multifactorial nature of metabolic syndrome, relying on glucose alone offers an incomplete assessment of metabolic health.

Multiplexed Biomarker Monitoring for Metabolic Assessment

While continuous glucose monitoring has significantly advanced metabolic disease management, relying on a single biomarker provides only a limited view of complex metabolic processes. Metabolic syndrome involves multiple interconnected pathways, including purine metabolism, oxidative stress, and energy balance, which cannot be fully captured through a single analyte.

This limitation motivates the need for multiplexed biomarker monitoring, where multiple related analytes are measured simultaneously to provide a more comprehensive understanding of metabolic state. In particular, purine metabolism offers a relevant framework for such analysis.

Uric acid, the end product of purine metabolism in humans, is widely used as a clinical indicator of gout, cardiovascular disease, and metabolic dysfunction. However, its concentration alone does not fully reflect metabolic dynamics. Upstream intermediates such as xanthine provide complementary information on purine turnover and enzymatic activity, enabling a more complete picture of metabolic regulation [10, 11].

By monitoring both uric acid and xanthine, it becomes possible to distinguish between accumulation and active metabolic flux, which is particularly important for understanding conditions such as gout and related metabolic disorders. In addition, external factors such as alcohol consumption can further modulate purine metabolism, reinforcing the importance of capturing multiple interacting signals rather than relying on a single biomarker.

Despite this need, most existing wearable sensing platforms remain limited to single-analyte detection, restricting their ability to capture the interconnected nature of metabolic processes. This limitation is particularly critical for purine metabolism, where wearable uric-acid sensing has only recently emerged and xanthine sensing remains comparatively underexplored, especially for sweat-based applications [12–19]. To better understand these dynamics, it is necessary to monitor multiple related biomarkers simultaneously, along with local environmental parameters that influence sensor response. Table 1.1 summarizes key biomarker groups that should be measured together and highlights the limitations of current wearable systems in enabling continuous multiplexed monitoring.

Table 1.1: Interconnected biomarkers for multiplexed sweat monitoring. Uric acid, xanthine, alcohol, pH, and temperature provide complementary physiological information for system-level interpretation of metabolic state. [20, 21]

Physiological context	Key biomarkers	Role in system-level interpretation	Clinical standard	Key limitation in wearable systems
Purine metabolism	Uric acid (UA), Xanthine	• UA: end-product accumulation • Xanthine: upstream metabolite • Pathway-level insight	• Serum UA (blood) • Xanthine: HPLC (plasma/urine)	• UA-only sensing common • No upstream context • Limited metabolic interpretation
Lifestyle–metabolism interaction	Uric acid (UA), Xanthine, Alcohol	• Alcohol modulates purine metabolism • Elevates UA levels • Links behavior to biochemical response	• Blood alcohol concentration • Serum UA	• No integrated multi-analyte platforms • Exogenous and endogenous signals not captured together • Limited real-time correlation
Local biochemical environment	pH, Temperature	• Affects enzyme activity • Influences electrochemical response • Required for signal interpretation	• Blood pH • Core body temperature	• Environmental parameters often excluded • Signal drift and variability • Poor cross-analyte consistency

1.2 Sweat as a Platform for Continuous and Multiplexed Sensing

Sweat is an appealing biofluid for wearable monitoring because it is accessible at the skin surface and can, in principle, support continuous and multiplexed biochemical analysis without repeated blood draws. It contains a range of metabolites, electrolytes, and physiologically relevant small molecules that reflect systemic state, making it attractive for tracking complex metabolic processes over time [21–28]. The physiological basis of sweat secretion is also important in this context because gland type, body location, and skin-surface lipid composition influence both secretion composition and sampling availability, as summarized in Fig. 1.1 [29–32].

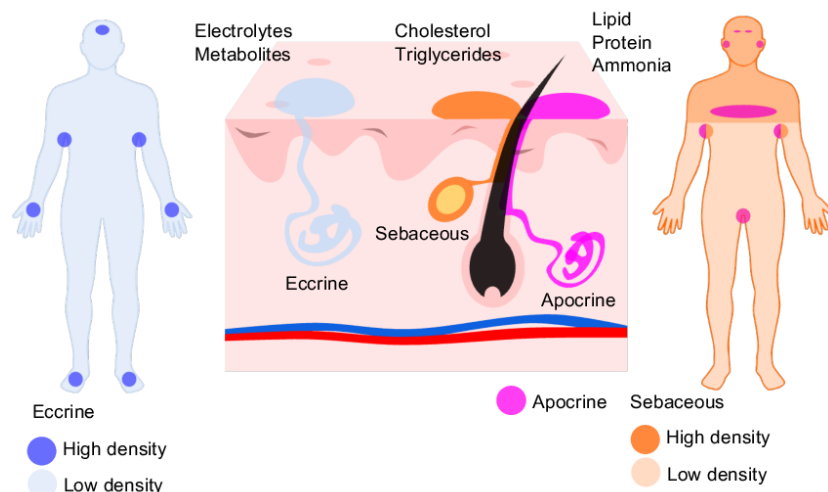


Figure 1.1: Schematics of eccrine, apocrine, and sebaceous glands and regional gland distribution in the human body. Adapted from Ref. [2] under the terms of the CC-BY license. Copyright 2026 The Authors, published by Wiley-VCH GmbH.

At the same time, the value of sweat extends beyond single-analyte readout. When paired with integrated microfluidic and electrochemical systems, sweat provides a potential platform for simultaneous monitoring of multiple interacting biomarkers within the same wearable format. Recent platforms have demonstrated this breadth across exercise and sports monitoring, nutrient and metabolite sensing, stress-hormone tracking, and noninvasive drug monitoring [33–49]. This makes sweat especially relevant for metabolic syndrome, where comprehensive assessment depends on capturing relationships across pathways rather than isolating one signal at a time. Representative sweat constituents and their sampling-relevant characteristics are summarized in Table 1.2.

Table 1.2: Representative sweat biomarkers, glandular origins, and sampling considerations. Adapted from Ref. [2] under the terms of the CC-BY license. Copyright 2026 The Authors, published by Wiley-VCH GmbH.

Biomarker	Class	Dominant Gland / Source	Sweat or Skin-Surface Concentration Range	Blood Concentration Range	Sampling-Relevant Notes
Sodium (Na^+)	Electrolyte	Ecrrine	~10–90 mM	~135–145 mM	Strong sweat-rate dependence due to ductal reabsorption [50, 51]
Chloride (Cl^-)	Electrolyte	Ecrrine	~10–80 mM	~95–105 mM	Closely coupled to Na^+ transport; sweat-rate dependent [50, 52]
Potassium (K^+)	Electrolyte	Ecrrine	~3–15 mM	~3.5–5.0 mM	Less sweat-rate dependence than Na^+/Cl^- [50]
Lactate	Metabolite	Ecrrine	~5–50 mM	~0.5–2 mM	Produced locally in glands; limited blood correlation [53]
Urea	Metabolite	Ecrrine	~2–20 mM	~2.5–7.8 mM	Diffuses from blood; enriched in sweat [32]
Ammonia	Nitrogenous	Ecrrine	~0.1–5 mM	~20–50 μM	pH-dependent; partially generated from urea breakdown [32, 53]
Glucose	Metabolite	Ecrrine	~0.01–0.5 mM	~4–6 mM	Low concentration; sensitive to contamination and sweat rate [23]
Uric acid	Purine metabolite	Ecrrine	~5–60 μM	~150–450 μM	Flow-dependent; influenced by skin surface accumulation [54]
Cortisol	Hormone	Mixed (ecrrine + surface partitioning)	~1–20 ng mL^{-1}	~50–200 ng mL^{-1}	Region-dependent; delayed relative to plasma [43]

Continued on next page

Table 1.2: Representative sweat biomarkers, glandular origins, and sampling considerations (continued).

Biomarker	Class	Dominant Gland / Source	Sweat or Skin-Surface Concentration Range	Blood Concentration Range	Sampling-Relevant Notes
Testosterone	Steroid hormone	Apocrine-associated	$\sim 0.1\text{--}10\text{ ng mL}^{-1}$ (axillary sweat)	$\sim 300\text{--}1000\text{ ng dL}^{-1}$	Site-specific enrichment in apocrine regions [55]
Androstenol / pheromone derivatives	Steroid derivatives	Apocrine	$\text{nM--}\mu\text{M}$	Low / variable	Secreted as odor precursors; microbially transformed [56, 57]
Free fatty acids	Lipids	Sebaceous (surface)	$\mu\text{g cm}^{-2}$ (skin surface)	—	Mix with sweat post-secretion; alter surface wetting [29]
Squalene	Lipid	Sebaceous	$\sim 5\text{--}15\%$ of sebum lipids	—	Contributes to hydrophobic skin-surface film; influences evaporation and partitioning [29]

Values represent literature-reported ranges or representative values under specific experimental conditions. Reported concentrations may vary depending on sweat rate, body location, and sampling methodology.

1.3 Challenges in Sweat-Based Sampling and Transport

Despite their clinical importance, continuous and non-invasive in situ monitoring of metabolic biomarkers in sweat remains challenging. For some targets, including triglycerides and ketones, the sensing chemistry itself is difficult because these reaction schemes can depend on externally supplied cofactors such as ATP and NAD^+ , which complicates direct deployment in sweat; ATP-dependent schemes, for example, rely on a molecule that is normally synthesized and stored intracellularly rather than being freely available at the sensing interface [58]. More broadly, however, the central bottlenecks are system-level: sweat must be generated on demand, transported under very low flow-rate conditions, refreshed continuously, and interfaced with sensors that remain stable over time [59, 60].

Table 1.3: Comparison of sweat induction methods and implications for wearable sampling. Adapted from Ref. [2] under the terms of the CC-BY license. Copyright 2026 The Authors, published by Wiley-VCH GmbH.

Induction Method	Sweat-Rate Regime	Spatial Localization	Temporal Control	Composition Dependence	Suitability for Long-Duration Wear
Exercise whole-body heating	/ High; can exceed several $\text{mg cm}^{-2} \text{ min}^{-1}$	Whole body	Low (physiology-driven)	Strong sweat-rate dependence due to ductal reabsorption; pH and metabolites influenced by systemic physiology	Limited; requires sustained activity or thermal stress [51, 71]
Localized thermal stimulation	Moderate; lower than exercise but sufficient for regional sampling	Localized	Moderate (controlled heating)	Composition remains sweat-rate dependent; partially decoupled from systemic physiology	Moderate; compatible with wearable integration [72, 73]
Cholinergic iontophoresis (e.g., pilocarpine, carbachol)	Low-moderate; sustained but localized	Highly localized	High (programmable stimulation)	Drug-dependent effects; intermediate electrolyte levels; possible pH shifts due to stimulation conditions	High; widely used for wearable and clinical applications [61, 74]

Sweat Induction and Availability

Sweat availability is inherently dependent on physiological state and external conditions, making it a primary constraint for wearable sweat sensing. Unlike blood or interstitial fluid, sweat is not continuously accessible and often requires active stimulation to generate sufficient volume for analysis [61–68]. Related noninvasive epidermal and touch-based platforms further illustrate broader routes for ambulatory biochemical access, including solid-state epidermal biomarkers and blood-free fingertip sensing [69, 70]. As a result, the choice of induction strategy plays a critical role in determining both the magnitude and temporal profile of sweat secretion. Table 1.3 summarizes major sweat-induction approaches and their implications for wearable sampling.

Common approaches to induce sweating include physiological activation, such as exercise and heat exposure, as well as externally controlled methods such as iontophoresis. Physiological stimulation can produce high sweat rates during active conditions, but is often transient and dependent on sustained activity or environmental factors. In contrast, iontophoresis enables localized and programmable sweat induction by delivering cholinergic agents directly to eccrine sweat glands, providing greater control over timing and location of secretion. Table 1.4 compares representative cholinergic agents used for pharmacologic sweat induction and highlights why

Table 1.4: Comparison of cholinergic agents for pharmacologic sweat induction. Adapted from Ref. [2] under the terms of the CC-BY license. Copyright 2026 The Authors, published by Wiley-VCH GmbH.

Drug	Mechanism of Action	Relative Sweat Output	Effective Penetration / Depth	Key Advantages	Limitations
Pilocarpine	Direct cholinergic agonist activating eccrine secretory cells	Moderate–high; transient	Dermal eccrine gland level	Clinically validated; reproducible response; established safety profile	Transient stimulation (~20–30 min under standard protocols) [52, 75]
Carbachol	Synthetic cholinergic agonist resistant to acetylcholinesterase degradation	High; more sustained than pilocarpine	Dermal eccrine gland level	Longer-lasting stimulation; effective at lower doses	Less clinically standardized; potential receptor desensitization with prolonged stimulation [61]
Acetylcholine (ACh)	Endogenous cholinergic neurotransmitter rapidly degraded by acetylcholinesterase	Low–moderate; rapid decay	Dermal eccrine gland level with limited diffusion radius	Physiologically native; rapid degradation enables precise temporal control	Rapid enzymatic degradation; short duration; rarely used alone [76]
ACh analogs / cholinergic esters	Synthetic cholinergic agonists engineered for enhanced stability and modified degradation kinetics	Variable; tunable	Dermal eccrine gland level	Adjustable degradation kinetics; potential for tailored stimulation profiles	Limited clinical validation; regulatory uncertainty [61, 77]

carbachol was selected in this work: relative to standard pilocarpine stimulation, carbachol is more resistant to enzymatic degradation and can provide longer-lasting localized sweat generation, which is advantageous for extending the usable sampling window.

Despite these differences, a key limitation shared across all induction strategies is the time-dependent decay of sweat rate following stimulation. While high sweat rates can be achieved during the active stimulation phase, secretion typically decreases once stimulation ceases, transitioning into a low-flow regime. This behavior is observed across both physiological and iontophoretic methods, although the rate and duration of decay may differ.

Sweat generation consists of an active stimulation phase followed by a decay in secretion over time. While physiological stimulation (e.g., exercise, heat) produces high sweat rates during activity, iontophoresis-based methods can extend the duration of secretion. However, all approaches ultimately transition into a low-flow regime. The dashed line indicates the minimum flow required for reliable sensing, highlighting that the effective sensing window is limited by decreasing sweat rates.

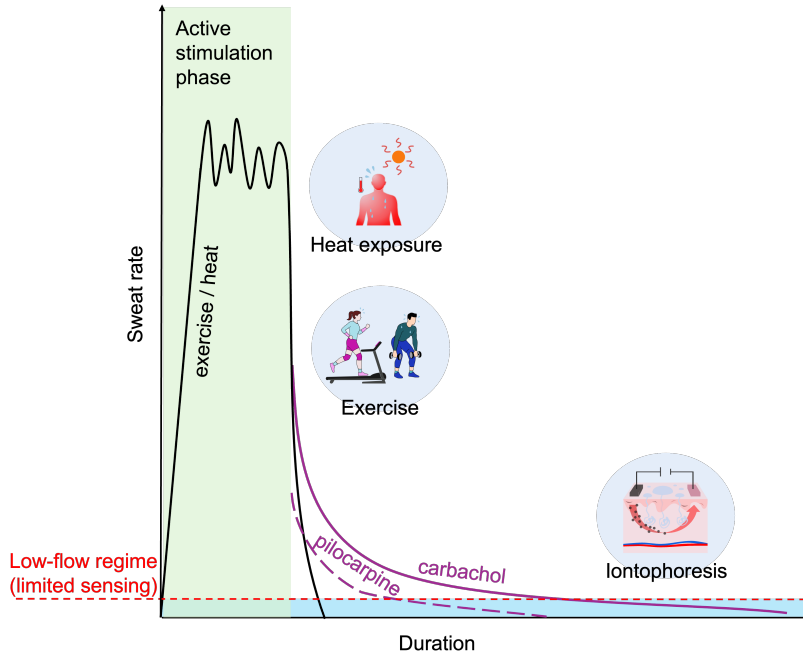


Figure 1.2: Schematic sweat-rate profiles for physiological stimulation and iontophoresis.

As illustrated in Fig. 1.2, although methods such as iontophoresis using agents like carbachol can prolong sweat secretion compared to conventional stimulation, the resulting sweat rate still decreases over time and eventually approaches levels that are insufficient for reliable sensing. This distinction highlights that extended duration of sweat production does not necessarily translate to extended sensing capability.

Consequently, the practical limitation of wearable sweat sensing is not solely determined by the ability to induce sweat, but by the ability to utilize the small volumes available during the low-flow regime. Under these conditions, evaporation, stagnation, and insufficient transport can significantly reduce the amount of usable sample reaching the sensing region.

This limitation defines an effective sensing window, corresponding to the period during which sweat flow remains above the threshold required for reliable measurement. Extending this window, particularly under low sweat-rate conditions, is therefore critical for enabling continuous and real-world wearable sensing applications.

Sampling Challenges Under Low Sweat Rate

While induction defines when sweat becomes available, the ability to sustain meaningful measurement is governed by how that sweat is sampled and transported. Under

real-world conditions, especially outside exercise, sweat rates are often low, transient, and spatially non-uniform, making fluid availability the dominant limitation for continuous monitoring rather than sensing chemistry alone.

At low secretion rates, several challenges emerge. First, insufficient sweat flux can lead to incomplete filling of microfluidic structures, limiting sensor activation and reproducibility. Second, slow transport increases residence time within the device, resulting in accumulation, mixing, and potential signal lag. Third, evaporation at the skin interface can compete with fluid entry, further reducing effective sampling volume.

Traditional wearable designs, which rely on passive wicking or symmetric capillary flow, are often effective only under high sweat-rate conditions. In low-flow regimes, these approaches can lead to stagnation, backflow, or delayed refreshment, preventing accurate temporal tracking of biochemical changes.

Recent advances in microfluidic architectures aim to address these limitations through engineered fluid management. Strategies such as capillary-enhanced inlets, wettability gradients, and asymmetric channel geometries enable more efficient sweat capture and directional transport. In particular, bioinspired designs incorporating surface-energy gradients and structural asymmetry have demonstrated improved fluid handling under low and variable secretion conditions.

These developments highlight a critical shift in wearable sweat sensing: rather than assuming continuous fluid availability, systems must be explicitly designed to operate under constrained and dynamic flow conditions [21, 22, 59, 60, 78, 79]. Efficient sampling under low flux is therefore a prerequisite for reliable continuous monitoring.

Need for Multiplexed Sensing in Metabolic Monitoring

Beyond fluid availability, the clinical utility of sweat sensing depends on the ability to capture meaningful biochemical information. Many existing wearable systems focus on single-analyte detection, such as glucose or lactate, which provides limited insight into complex physiological states.

Metabolic disorders, including conditions related to purine metabolism and oxidative stress, are inherently multifactorial. For example, biomarkers such as uric acid, xanthine, and related metabolites are interconnected within metabolic pathways and cannot be fully interpreted in isolation. Monitoring a single analyte may therefore fail to capture the underlying physiological dynamics.

Sweat contains a broad range of detectable biomarkers, including electrolytes, metabolites, and small molecules, many of which reflect both systemic and local physiological processes [23–25]. However, their concentrations and dynamics are influenced by sweat rate, glandular origin, and surface interactions, further emphasizing the need for integrated and contextual measurement.

Multiplexed sensing platforms enable simultaneous monitoring of multiple analytes, allowing for more comprehensive assessment of metabolic states [21, 79–81]. When combined with continuous sampling, such systems provide the potential to track dynamic biochemical changes over time rather than relying on isolated measurements.

On-body Validation and System-Level Considerations

Laboratory demonstrations under controlled conditions often do not fully capture the challenges associated with real-world operation [36, 74, 78]. Therefore, evaluating performance of wearable sweat sensors under on-body conditions is essential, where physiological variability, environmental factors, and user behavior introduce additional complexity.

On-body testing highlights several critical considerations. First, sweat generation is not continuous and may require periodic induction, leading to discrete sensing windows. Second, sweat rate decreases over time following stimulation, affecting both signal stability and measurement duration. Third, motion, temperature, and skin heterogeneity can influence fluid transport and sensor response.

These factors underscore the importance of system-level integration. Effective wearable platforms must coordinate induction, sampling, transport, and sensing to maintain reliable performance over time. In particular, the ability to operate under low sweat rates, extend the usable sensing window, and reduce the frequency of induction sessions is essential for practical deployment.

By validating performance in on-body settings, wearable systems can be assessed not only for sensing accuracy but also for their ability to sustain continuous operation under realistic conditions. This system-level perspective is critical for translating laboratory advances into meaningful applications.

Sensor Integration Challenges

Even if sweat can be induced and transported reliably, integration with the sensing interface remains nontrivial. Long-duration operation raises concerns about fouling, drift, and loss of electrochemical or biochemical stability over time. These issues

become especially important when devices are expected to operate continuously under skin-contact conditions.

Additional complexity arises when multiple biomarkers must be monitored simultaneously. Multiplexed sensing requires compatible transport paths, stable local chemical environments, and sufficient sample delivery to each sensing region. Skin secretions other than eccrine sweat, including sebum and apocrine components, can further complicate sensor performance by introducing contamination and interfacial instability.

1.4 Existing Approaches and Limitations

Existing wearable sweat platforms have made substantial progress in sensing chemistry and device integration, but many remain primarily sensing-focused rather than transport-engineered [21–24, 82, 83]. As a result, their performance is often strongest under favorable sweating conditions and becomes less reliable in the low-flow regime most relevant to sedentary, real-world monitoring.

Absorbent Patches

Absorbent and paper-based patches offer simple, low-cost sweat collection, but they generally depend on accumulation over time rather than controlled transport [22, 84–87]. This can reduce temporal resolution, increase evaporation losses, and make it difficult to distinguish fresh sweat from previously collected fluid.

Microfluidic Devices

Microfluidic sweat devices provide better compartmentalization and can support routing, timing, and multiplexed analysis [21, 80, 81, 88–91]. However, many such systems still rely on sufficiently high sweat rates or sustained hydrostatic pressure to fill channels and reservoirs. Under low-flow conditions, incomplete filling, dead volume, backflow, and poor directional control remain major limitations.

Epidermal Electronics

Epidermal electronic platforms enable intimate skin contact and highly integrated sensing, but they do not by themselves solve the fluid-handling problem [21, 83]. Without a sampling layer designed for low-rate transport and refreshment, signal quality can still be limited by unstable sweat access, local depletion, and inconsistent renewal at the sensing interface.

Sweat Induction and Effective Sensing Window

Localized sweat induction using cholinergic agents such as carbachol is widely used to enable on-demand sweat generation in wearable sensing systems [61–65, 91, 92]. Compared with conventional stimulants, carbachol can produce prolonged localized sweating and sustain secretion for extended periods following a short iontophoresis session.

However, prolonged sweat availability does not necessarily translate to sustained sensing performance. After iontophoretic stimulation, sweat secretion exhibits a time-dependent decay, with the initial high secretion rate decreasing to substantially lower levels over time [61]. Under these low secretion conditions, the available sweat volume becomes increasingly susceptible to evaporation, stagnation, and mixing of old and newly secreted fluid.

As a result, the effective sensing window, defined as the duration over which sweat can be reliably sampled and analyzed, is often significantly shorter than the total stimulation duration. Conventional wearable systems, which typically rely on higher flow rates for stable operation, are therefore unable to fully utilize the extended sweat production enabled by carbachol.

To compensate for this limitation, repeated iontophoresis sessions are often required to maintain adequate sampling conditions, reducing practicality for continuous and real-world monitoring. These challenges highlight the need for systems capable of efficiently capturing and transporting sweat under low secretion rates, in order to maximize the usable output from each induction event and reduce reliance on frequent stimulation.

Across these approaches, several limitations recur: many systems require relatively high sweat rates, operate only for limited durations, provide weak control over flow direction, and still depend on frequent induction to maintain sampling. In this sense, current systems are often optimized for sensing once sweat is available, rather than for engineering reliable sweat transport under realistic low-flow conditions. This gap directly motivates the transport-centered strategy developed in this work.

1.5 Thesis Objective and Scope

The overarching objective of this thesis is to address the following question: how can we enable continuous, non-invasive, and multiplexed chemical sensing under low and intermittent sweat conditions? This question is motivated by three connected limitations in current wearable sweat-sensing systems.

First, sweat availability and transport remain major bottlenecks. Sweat rates are often low and intermittent outside high-exertion settings, and limited driving force can prevent small volumes of sweat from entering and refreshing the sensing region. This thesis therefore focuses on microfluidic strategies that improve sweat harvesting, directional transport, and outlet-mediated refreshment under constrained flow conditions.

Second, existing wearable platforms often have limited sensing capability. Many systems still rely on single-analyte detection, which restricts their ability to provide multiplexed chemical information for physiological interpretation. This work therefore integrates electrochemical sensing modules for simultaneous monitoring of multiple metabolically relevant biomarkers together with local correction parameters.

Third, wearable sweat sensors require stronger demonstration in real-world and clinical contexts. Many reported systems are evaluated primarily under controlled laboratory conditions, with limited evidence for multi-day operation, low-flow performance, or clinically relevant monitoring. This thesis addresses this gap by validating the integrated platform during on-body studies, including daily activity and gout-related metabolic monitoring.

Together, the scope of this thesis spans sweat availability and transport, multiplexed sensing capability, and real-world validation. These efforts establish design principles for continuous and noninvasive metabolic monitoring based on sweat.

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Chapter 2

BIOINSPIRED MICROFLUIDIC SWEAT SAMPLING SYSTEM

Materials from this chapter appear in “Shin, Soyoung, Ruixiao Liu, Yiran Yang, José A. Lasalde-Ramírez, Gwangmook Kim, Chihyeong Won, Jihong Min, Canran Wang, Kexin Fan, Hong Han, Chibuikwe Uwakwe, Wenzheng Heng, Tzung K. Hsiai, Zhaoping Li, John D. FitzGerald, and Wei Gao (Aug. 2025). ‘A bioinspired microfluidic wearable sensor for multiday sweat sampling, transport, and metabolic analysis’. In: *Science Advances*.” [1]

2.1 Design Principles

The microfluidic system was designed to enable continuous sweat sampling under conditions of limited secretion by integrating bioinspired wettability and geometric features [2–15]. The design follows microfluidic transport principles in which capillary driving forces, viscous resistance, and interfacial wetting determine whether small liquid volumes can be collected and refreshed reliably [16, 17]. The device design is organized around three coupled functions: sweat harvesting, sweat transport, and outlet refreshment. Together, these functions convert weak and intermittent sweat input into directional flow through the sensing region.

Fig. 2.1 summarizes the Chapter 2 design concept. The first focus is the harvesting layer, which must collect very small sweat volumes at the skin interface and promote unidirectional vertical transfer into the device. The second focus is the transport layer, where the microchannel architecture must support fast, small-volume, unidirectional horizontal flow across the sensing area. The third focus is the outlet, which must sustain refreshment, stabilize directional flow, and reduce stagnation after the channel fills.

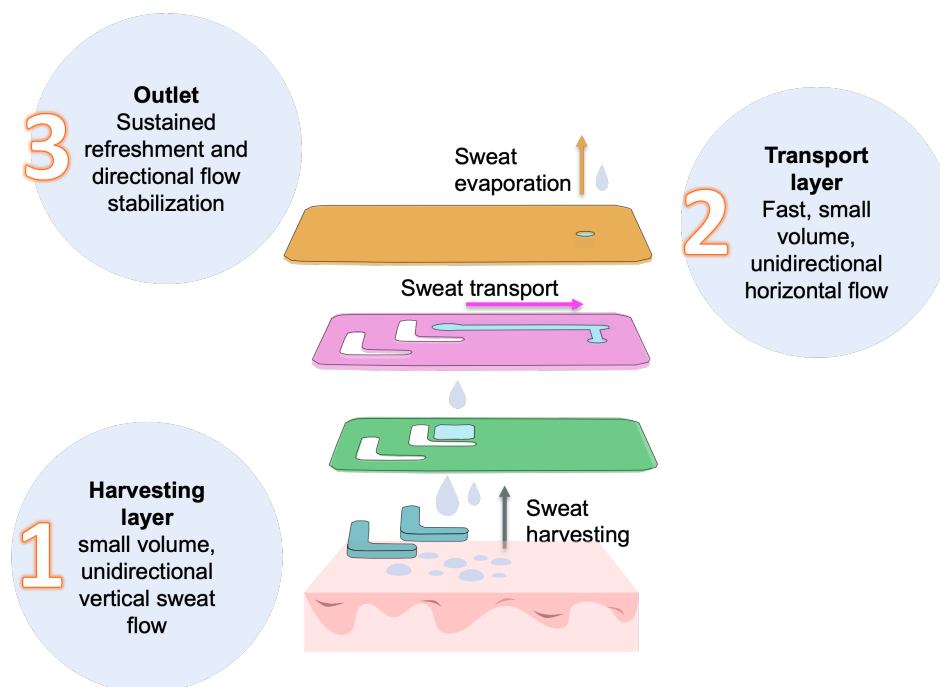


Figure 2.1: Bioinspired microfluidic architecture for continuous sweat sampling under low-flow conditions. The system is designed around three coupled functions: (1) a harvesting layer for small-volume, unidirectional vertical sweat flow from skin into the device, (2) a transport layer for fast, small-volume, unidirectional horizontal flow through the microchannel, and (3) an outlet for sustained refreshment and directional flow stabilization. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Specifically, a superhydrophobic–superhydrophilic Janus membrane was incorporated at the inlet to facilitate efficient sweat harvesting and unidirectional transport from the skin. This asymmetric wettability enables rapid absorption on the hydrophilic side while preventing evaporation and backflow on the hydrophobic side, thereby forming a stable liquid interface that maintains contact with the downstream microchannel. To further support transport, hierarchically graded microchannels inspired by pitcher plant trichomes were introduced [15]. These structures combine dual-height ribs and gradual width expansion to guide fluid movement through capillary pressure gradients. Finally, a Janus outlet was used to sustain downstream refreshment and stabilize directional flow by reducing fluid accumulation at the channel terminus. In this chapter, this integrated architecture is referred to as our bioinspired microfluidic sweat sensor system (BMS³). The inlet, microchannel, and outlet together establish a passive system that maintains forward sweat transport without reliance on external pumping.

From a fabrication standpoint, the Janus membranes were patterned from 50- μm -thick PET films and modified through a combination of silica nanoparticle coating and localized O_2 plasma treatment. In this scheme, the silica-based coating creates the hydrophobic background, while masked plasma exposure defines the hydrophilic regions used at the inlet and outlet. The hierarchical microchannels were fabricated separately on PI film by photolithographic patterning of SU-8 layers to create the dual-height rib geometry. This material combination was chosen to couple flexible membrane-based wettability control with robust microstructured channels that could preserve the designed capillary features during assembly and operation.

2.2 Mechanism of Directional Transport

Inlet Janus: Wettability Design and Meniscus Formation

The inlet Janus membrane was inspired by the lotus leaf because it provides a natural example of asymmetric wetting generated by surface structure. The upper surface of the lotus leaf contains hierarchical micro- and nanostructures that produce superhydrophobic behavior, whereas the lower surface is comparatively smoother and more hydrophilic. This contrast offers a useful model for designing a membrane that favors directional liquid transfer rather than uniform spreading. In our device, this biological concept was translated into a silica-treated hydrophobic region combined with a localized plasma-treated hydrophilic region, creating the wettability asymmetry needed for one-way sweat entry and stable interfacial transport [18, 19].

Fig. 2.2 summarizes the Janus membrane concept and fabrication workflow based on asymmetric wettability design. Fig. 2.2A presents the design concept: a wettability contrast derived from the lotus-leaf model is used to favor one-way transport from the hydrophilic inlet toward the downstream layer while limiting backward spreading on the hydrophobic side. Fig. 2.2B shows how this membrane was fabricated. A hydrophobic silica-nanoparticle coating was first formed on a 50- μm -thick PET substrate. The silica nanoparticles were fluorinated using PFOTS and dispersed in anhydrous ethanol, after which the PET membrane was dip-coated and dried to form a rough, low-surface-energy background layer. A mask was then placed over the membrane, and O_2 plasma was applied to the exposed circular area to generate the hydrophilic inlet while preserving the surrounding silica-coated region. This sequence produced the superhydrophobic–hydrophilic contrast needed to draw liquid into the plasma-treated inlet while limiting lateral spreading on the surrounding silica-coated surface.

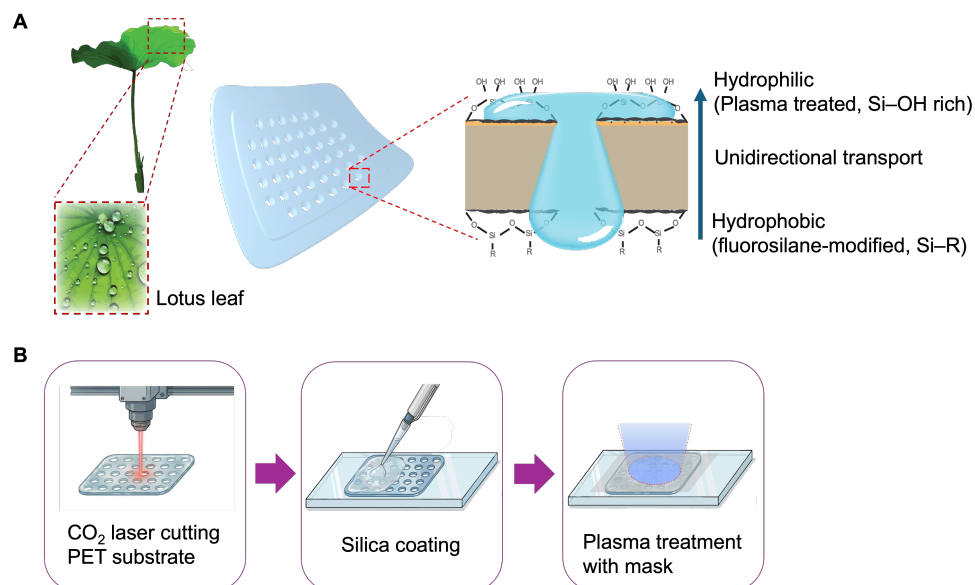


Figure 2.2: Janus membrane concept and fabrication. (A) Schematic of Janus membrane inspired by lotus leaf. (B) Fabrication method. Panel A adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

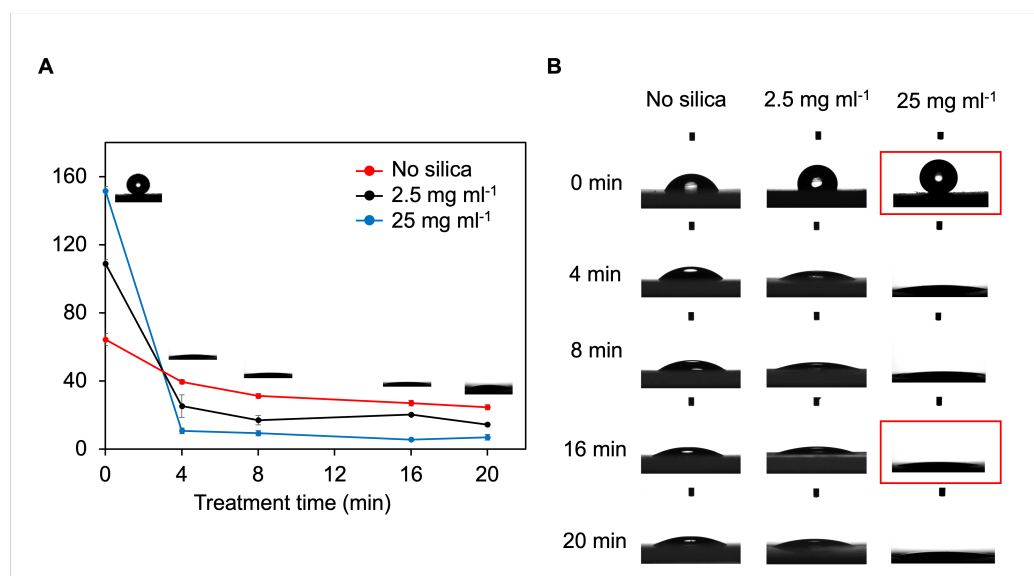


Figure 2.3: Selection of silica-treatment condition for Janus membrane fabrication. (A) Wettability and treatment-condition test of silica. (B) Contact-angle view. The red rectangle indicates the final condition. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Fig. 2.3 summarizes the selection of the silica-treatment condition for Janus mem-

brane fabrication. Fig. 2.3A compares the contact-angle values measured under the tested treatment conditions. Fig. 2.3B shows representative droplets for those same conditions. The condition marked by the red rectangle was selected because it produced the highest hydrophobicity while remaining compatible with subsequent patterning of the hydrophilic inlet. This choice established the surface state required to suppress lateral wetting outside the inlet region.

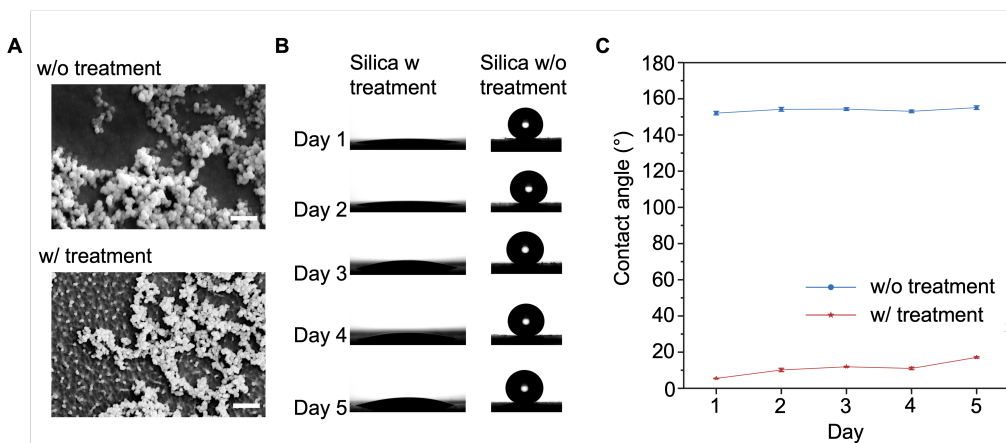


Figure 2.4: Surface morphology and wettability stability of the silica-treated membrane. (A) SEM comparison. (B) Contact-angle test for stability over multiple days. (C) Summary of stability over multiple days.

Fig. 2.4A shows the surface morphology of the silica layer by SEM. The untreated surface appears more aggregated and less uniform, whereas the silica-treated surface is more uniform and less aggregated. The scale bar in Fig. 2.4A is 100 nm. Fig. 2.4B then compares the contact-angle images over time, and Fig. 2.4C quantifies that stability over multiple days. These results show that the selected silica treatment provides both the surface morphology and the durable wettability contrast required for localized droplet formation at the inlet.

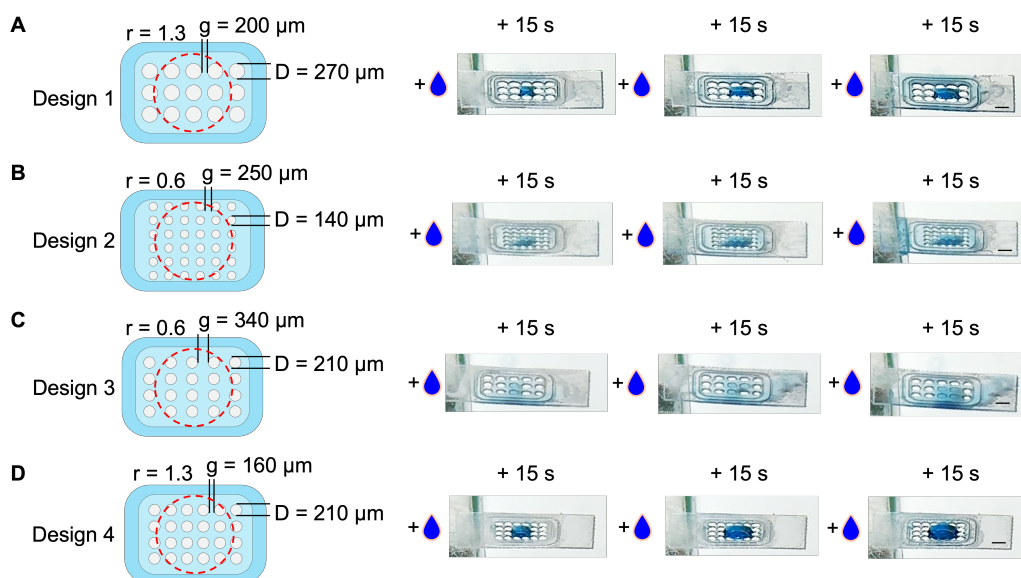


Figure 2.5: Comparison of meniscus formation of Janus inlet designs. (A) Design 1. (B) Design 2. (C) Design 3. (D) Design 4. Scale bars, 2 mm. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

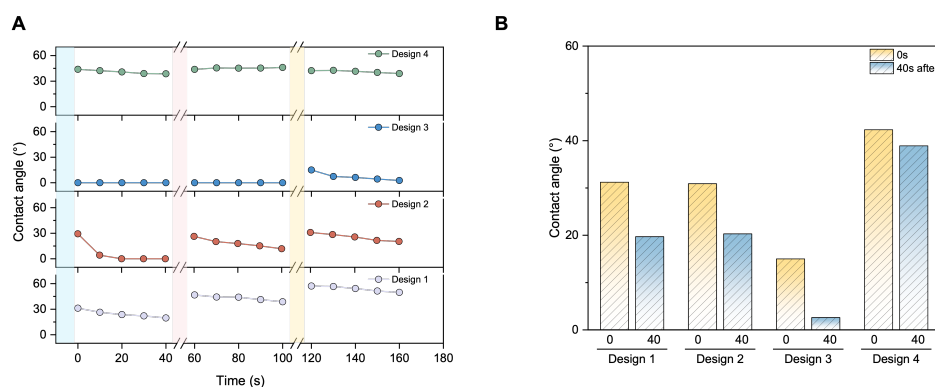


Figure 2.6: Contact-angle analysis of Janus inlet designs during repeated droplet addition. (A) Contact-angle analysis of meniscus formation comparison. (B) Meniscus stability test from 0 to 40 s. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

The first design criterion for the Janus inlet was the bridging effect between neighboring holes. Fig. 2.5A–D shows the four inlet designs and the corresponding droplet behavior during repeated 0.1 μL additions. In this comparison, a droplet delivered onto the patterned inlet should not remain confined to isolated holes; instead, adjacent menisci should merge to create a continuous liquid bridge across the inlet

region. This behavior is governed by the hole-to-gap ratio, $r = D/g$, where D is the hole diameter and g is the spacing between neighboring holes. When $r < 1$, the gaps are wider than the holes, so neighboring droplets remain separated and bridging is difficult. By contrast, when $r > 1$, the gaps are sufficiently small relative to the hole diameter that adjacent menisci overlap more readily, making continuous connection easier to establish. For this reason, $r > 1$ is desirable for stable bridging and sustained inlet operation.

Fig. 2.5C, corresponding to Design 3 with $r = 0.6$ ($D = 210$ m, $g = 340$ m), shows limited droplet bridging and weak continuity during repeated addition. Fig. 2.5D, corresponding to Design 4 with $r = 1.3$ ($D = 210$ m, $g = 160$ m), shows more reliable formation of a continuous liquid pathway and better retention of a localized droplet. These results indicate that reliable bridging requires sufficiently close spacing relative to the opening diameter.

Once this bridging condition was established, the next issue was how capillarity influences the liquid volume required to maintain the bridge. Fig. 2.6A shows the contact-angle evolution during repeated droplet addition for the four inlet designs. Fig. 2.6B compares the initial and later contact angles for those designs. At fixed $r > 1$, the absolute values of D and g still affect meniscus curvature and therefore capillary pull. A moderate feature size, as in Design 4, produces a more curved meniscus and stronger capillary force, which helps pin the liquid locally and stabilize the bridge after each droplet addition. If the features become too large, the meniscus becomes flatter and the capillary pull weakens, so a larger liquid volume is required to sustain the same continuous connection. This behavior is seen by comparing Design 4 ($D = 210$ m, $g = 160$ m) with Design 1 ($D = 270$ m, $g = 200$ m), both of which satisfy $r = 1.3$. Although both designs meet the bridging criterion, Design 4 maintained a more stable contact-angle response during repeated addition, whereas the larger-feature design showed weaker retention and greater fluctuation. Reliable Janus inlet operation therefore requires both a sufficiently large hole-to-gap ratio to enable bridging and sufficiently strong capillary confinement to preserve that bridge under low-volume input conditions.

Based on these comparisons, Design 4 was selected as the final Janus inlet structure. This selected Janus membrane supports unidirectional liquid transport rather than simple gravity-driven leakage. When water is introduced from the hydrophilic side, the droplet spreads, bridges the patterned opening, and is transported through the membrane along the intended direction. By contrast, reversing the orientation does

not produce uncontrolled backflow; instead, the wetting asymmetry still biases the liquid pathway so that the Janus membrane maintains directional transport. This result is important because it shows that the inlet behavior is governed by interfacial design and capillary asymmetry, not merely by device orientation. Thus, the selected Design 4 membrane preserves unidirectional flow even when the gravitational orientation is changed, which is important for practical wearable operation on a moving body.

Microchannel Transport: Phase Definition and Continuous Flow

The microchannel design in this work was inspired by the trichomes on the inner surface of the pitcher plant peristome, which are known to support extremely rapid liquid transport. In this natural system, droplets do not simply hop from one isolated site to another; instead, they spread into a thin continuous film that is guided along anisotropic surface features. This film-based transport is much faster than that observed in other bioinspired liquid-guiding systems such as spider silk or cactus spines, underscoring the efficiency of the pitcher-plant strategy for directional capillary motion [15]. The key principle is hierarchical microstructuring: multi-scale grooves and ribs promote film formation, maintain capillary connectivity, and bias liquid movement along the preferred direction. This mechanism provided the biological basis for our own microchannel design.

Based on this principle, the artificial microchannel was designed with hierarchical rib structures and a lateral width gradient to reproduce the capillary guidance effect of pitcher-plant trichomes. Fig. 2.7 summarizes this concept and the associated fabrication strategy. Fig. 2.7A relates the pitcher-plant trichome motif to the engineered channel geometry, and Fig. 2.7B outlines the fabrication sequence used to realize the microstructured architecture.

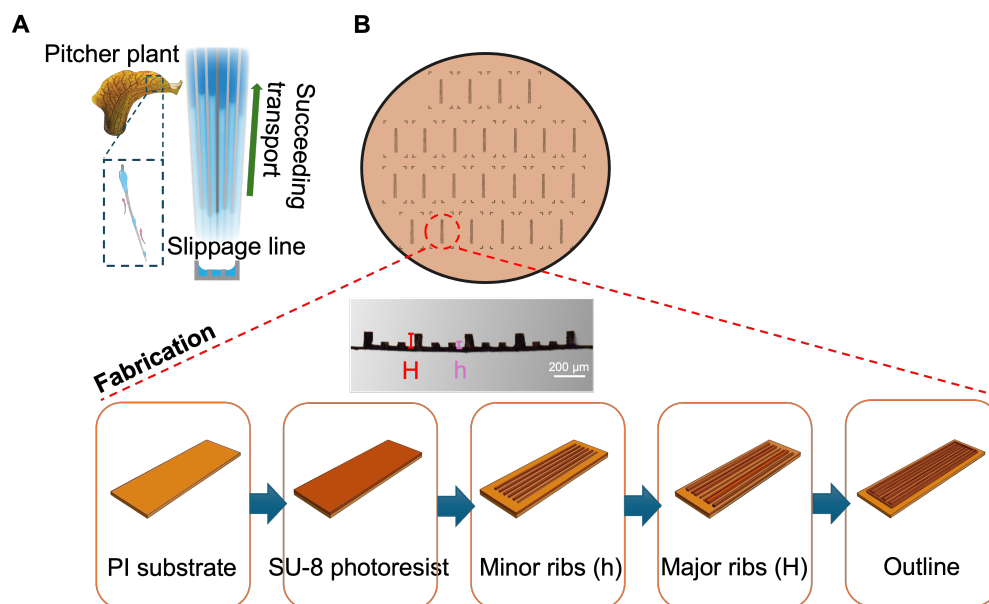


Figure 2.7: Overview and fabrication of the bioinspired microchannel system. (A) Overview of microchannel inspired by pitcher plant trichome. (B) Fabrication. Panel A adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

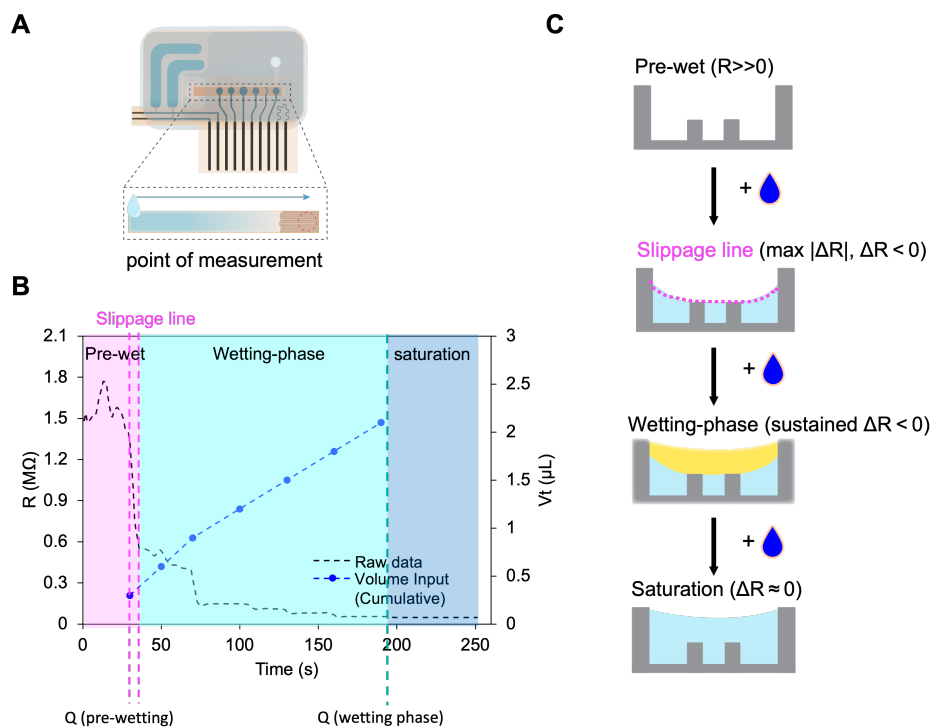


Figure 2.8: Definition of microchannel transport phases using impedance monitoring. (A) Schematic of impedance test with flow stages. (B) Impedance graph used to define each flow stage and the flow rates for pre-wetting and wetting phase. (C) Description of each flow stage from pre-wet to saturation. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Once fluid enters the microchannel, its transport can be divided into four regimes using impedance measurements and cumulative volume input, as summarized in Fig. 2.8. Figure 2.8A provides the schematic framework for this interpretation by illustrating how fluid progression through the channel is tracked together with the corresponding flow stages. Figure 2.8B shows the representative impedance trace used to define these regimes and to extract the pre-wetting and wetting-phase flow rates. Figure 2.8C summarizes the physical meaning of each stage from pre-wet to saturation. High resistance indicates that the channel is either unfilled or only weakly connected by liquid. During the pre-wet phase, resistance remains high because liquid has not yet formed a continuous path across the channel. As fluid accumulates, the system reaches the slippage transition, where the slippage line forms and resistance drops sharply. This abrupt drop marks the onset of continuous hydraulic connection. After this transition, the channel enters the wetting phase, during which liquid continues to move through the structure and resistance changes

more gradually as previously dry regions become connected. When transport finally ceases—likely because outlet-driven pumping becomes insufficient or the channel approaches saturation—the resistance reaches a plateau.

The flow-rate metrics used below are defined directly from Fig. 2.8B. The pre-wetting flow rate, Q_{pre} , is the average volumetric input rate required to reach the slippage line; that is, it is the cumulative input volume divided by the time required for slippage-line formation. The wetting-phase flow rate, Q_{wet} , is the average flow rate during the wetting phase after the slippage line has formed. These two quantities separate the initial establishment of continuous flow from the later ability of the channel to sustain transport. Performance across different designs was evaluated using the slippage condition together with these two flow-rate metrics. Specifically, Q_{pre} describes the average rate required to reach slippage-line formation, and Q_{wet} describes the average rate during the wetting phase after slippage-line formation. This separation allows designs that trigger slippage early but fail to sustain downstream transport to be distinguished from designs that perform well in both regimes.

Microchannel Geometry: Design Optimization and Performance

Because transport in this microchannel is driven passively, capillary pressure is the primary force that determines whether liquid merely fills locally or propagates rapidly along the structure. We therefore focus on how channel geometry generates a capillary-pressure gradient and, in turn, accelerates transport. Figure 2.9A visualizes the key comparison between a constant-width channel (Design 1, W_1) and a laterally graded channel (Design 2, W_2). In the constant-width geometry, the capillary driving force remains nearly uniform along the channel and therefore produces little net pressure gradient ($\Delta P = 0$). In contrast, the graded geometry produces a positive capillary-pressure gradient ($\Delta P > 0$), which drives rapid film formation and directional liquid propagation. Figure 2.9B then shows the corresponding flow-rate measurements, confirming that the width-gradient design produces faster transport.

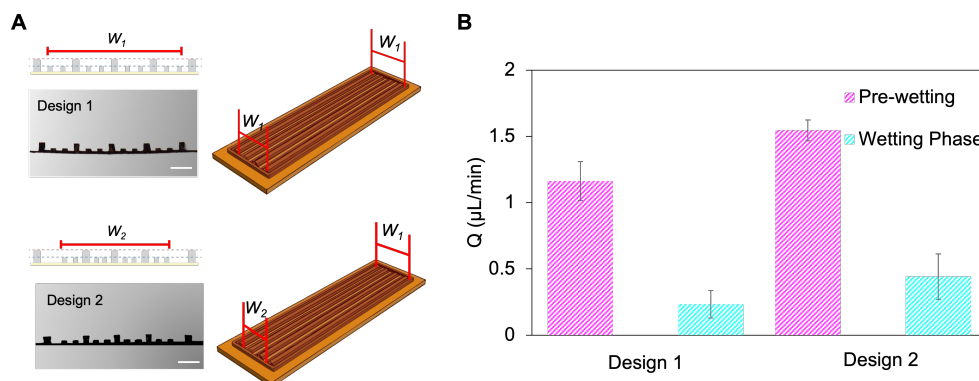


Figure 2.9: Capillary-pressure gradient induced by channel-width variation. (A) SEM and schematic of width-gradient effect for Design 1 and Design 2. Scale bar, 200 μm . (B) Flow-rate result for capillary-pressure gradient. Error bars represent s.d. of the mean from 3 samples ($n = 3$). Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

As summarized in Fig. 2.9B, introducing a width gradient improves transport in two distinct ways. First, the capillary-pressure gradient increases Q_{pre} , so the thin liquid film forms more quickly and the slippage line is reached earlier. Second, the same gradient increases Q_{wet} after the slippage transition, so liquid continues to advance more rapidly through the channel and refreshment is improved. In contrast, the constant-width channel lacks this directional capillary bias, leading to slower pre-wet filling and weaker wetting-phase transport. These results show that channel-width variation is a central design feature for accelerating both the onset and the persistence of microchannel transport.

With the width-gradient effect established, we next examined the role of rib height using Design 2 as the reference geometry. Relative to Design 2, Design 3 increases the major rib height (H), whereas Design 4 increases the minor rib height (h). As shown in Fig. 2.10A, increasing H in Design 3 creates deeper pockets and gaps, which produces a more strongly curved slippage line. Because of that deeper curvature, more liquid volume is required before the slippage line can form, so the onset of continuous transport is delayed. This slows the system overall in both the pre-wet and wetting phases, with the penalty especially pronounced during the wetting phase because the deeper curved meniscus also makes the downstream flow less stable.

Increasing the minor rib height in Design 4 causes a different but similarly unfavorable effect. In this case, the slippage line becomes less distinct and also requires

more liquid volume to form, indicating delayed thin-film formation. The resulting pre-wet flow is even slower than that of Design 3, and once transport begins, the flow is less directional and less continuous. Consequently, Design 4 also shows a reduced wetting-phase flow rate. The Design 2-4 comparison therefore indicates that the reference geometry best balances slippage-line formation, directional continuity, and sustained wetting-phase transport.

Design 2 was selected because it combines early slippage with strong Q_{wet} , indicating both efficient transition to continuous flow and sustained downstream propagation under limited input conditions. In the selected design, slippage begins at a small cumulative volume input of approximately 0.2 μL , indicating that only a minimal amount of fluid is required to establish continuous flow. The lateral width gradient further promotes forward movement by maintaining a directional capillary bias. Compared with constant-width channels, the graded geometry supports more uniform and sustained transport with less flow decay during operation.

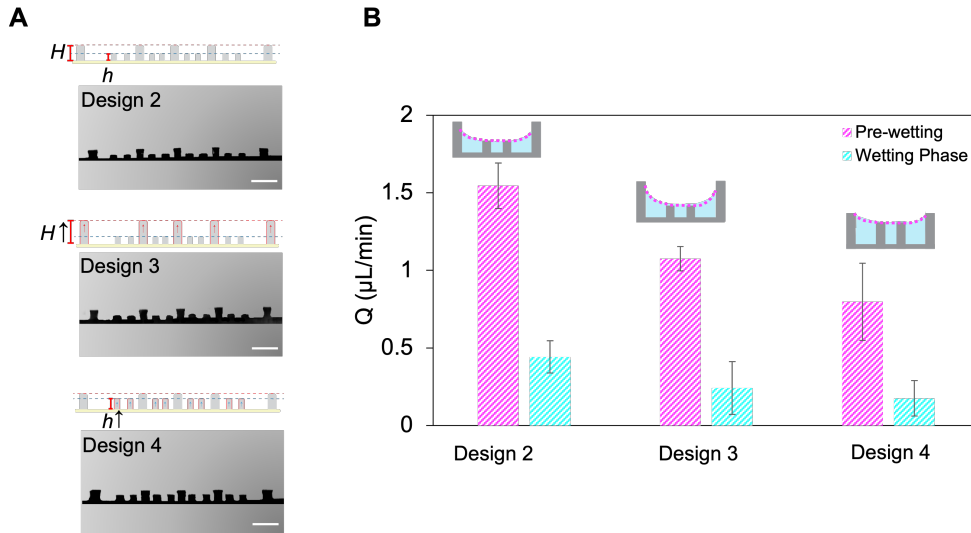


Figure 2.10: Effect of rib height on microchannel transport. (A) SEM and schematics of rib effect, with H for Design 3 and h for Design 4. Scale bar, 200 μm . (B) Flow-rate result. Error bars represent s.d. of the mean from 3 samples ($n = 3$). Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Outlet Janus: Flow Stabilization and Continuous Refreshment

As discussed in Section 1.6, the outlet is important because it must do more than simply release fluid: it must also suppress backflow and preserve the conditions

needed for continued transport. To test the specific role of Janus behavior at the outlet in a simple and direct way, the outlet structure in Fig. 2.11A was evaluated using the same gap (g) and diameter (D) condition selected for the inlet. This choice minimizes additional geometric variables and allows the comparison to focus on whether the Janus outlet itself improves downstream removal. Figure 2.11A compares the flow-speed test with and without outlet Janus. Figure 2.11B compares the corresponding flow-stability behavior. Without outlet regulation, fluid accumulates at the end of the channel, backpressure increases, and the flow rate decreases over time, indicating that sustained transport would otherwise require an additional driving force.

By contrast, the Janus outlet maintains a more stable flow after initial filling. This comparison shows that the outlet continuously relieves accumulated fluid and preserves forward transport. The outlet Janus therefore supports outlet refreshment and passive pumping. This is important because continuous sampling depends not only on fluid entry into the channel, but also on controlled downstream removal that suppresses stagnation and flow decay.

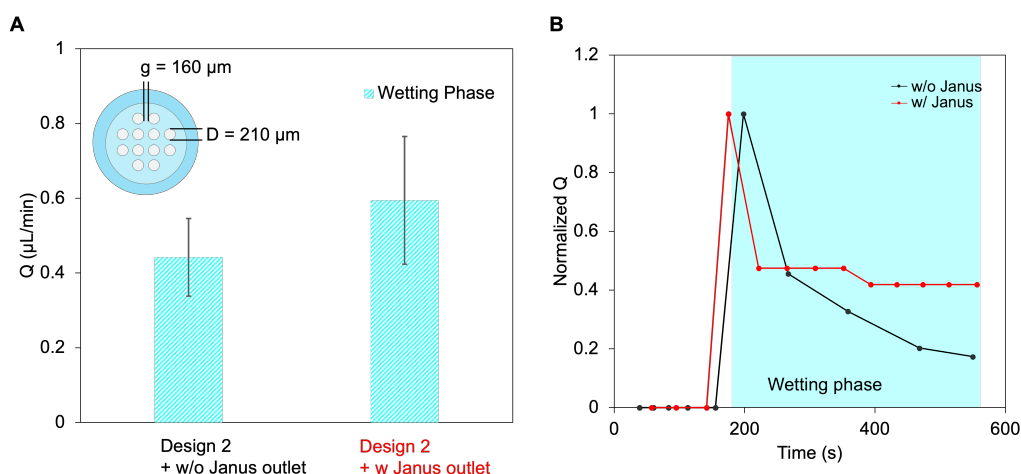


Figure 2.11: Flow-speed and flow-stability comparison with and without outlet Janus. (A) Flow-speed test comparison with and without outlet Janus using the same gap (g) and diameter (D) condition selected for the inlet. (B) Flow-stability test comparison with and without outlet Janus. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Integrated Transport Mechanism

The overall transport behavior arises from coordinated control across three regions. At the inlet, wettability patterning governs droplet formation and ensures efficient

transfer into the microchannel. Within the channel, structural design enables rapid transition to continuous flow and sustained propagation. At the outlet, asymmetric wettability maintains flow by enabling continuous fluid removal and preventing stagnation.

Through this integration of surface and structural design, the system supports directional and sustained fluid transport under low secretion conditions without external pumping.

2.3 System-Level Validation of Directional Sweat Sampling

To determine whether the bioinspired elements improve transport at the device level, we compared a control microfluidic patch lacking the Janus inlet/outlet and hierarchical channel features with our system (BMS³). Fig. 2.12 shows this comparison under stepwise dye addition, where each droplet corresponds to 0.5 μL .

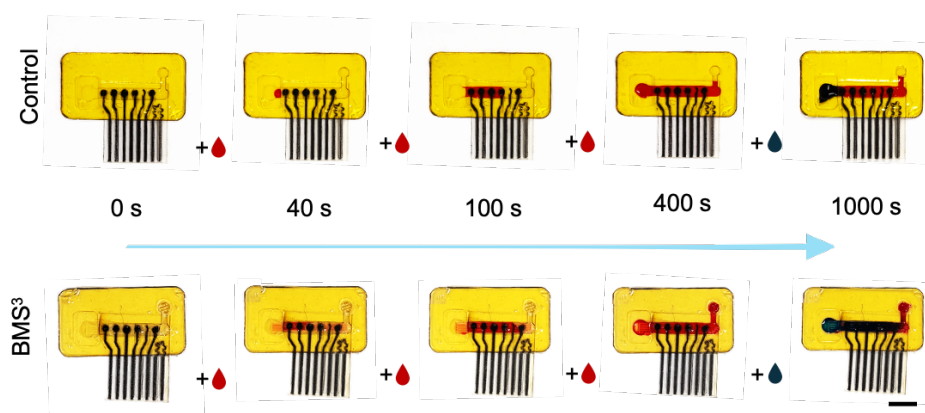


Figure 2.12: Flow transport comparison between control and integrated patches. Scale bar, 5 mm. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

This comparison directly illustrates the functional role of the integrated architecture. In the control device, newly added fluid accumulates locally and advances only after sufficient volume builds up, producing intermittent and nonuniform motion along the channel. In contrast, in BMS³, each added droplet is incorporated into an already connected liquid pathway, so the dyed fluid propagates more continuously from the inlet toward the outlet. This difference shows that the combined inlet, channel, and outlet design transforms transport from a buildup-driven process into sustained propagation.

This system-level behavior is consistent with the component-level measurements discussed in Section 2.2. As described above, the selected channel design forms the slippage line with a small input volume and maintains forward propagation during the wetting phase. Fig. 2.11 shows that the outlet Janus improves both flow speed and flow stability after filling by reducing fluid accumulation at the channel terminus. The whole-device comparison in Fig. 2.12 therefore confirms that these individual design advantages translate into improved transport across the full patch.

To evaluate the fully integrated device after the in vitro transport studies, we next established a system-level on-body flow test on human skin. In this experiment, sweat first entered the system through the Janus inlet. Once sweat had entered the device, a small amount of blue dye was added near the inlet region, at the first electrode, to visualize downstream transport within the assembled patch. As sweat continued to flow, the dye moved rapidly through the microchannel across the sensing region and was ultimately carried to the Janus outlet, where outlet refreshment could be observed. As shown in Fig. 2.13, this experiment bridges the in vitro characterization and the subsequent long-duration on-body studies by confirming that the assembled patch operates as a continuous sampling pathway on skin rather than as disconnected components.

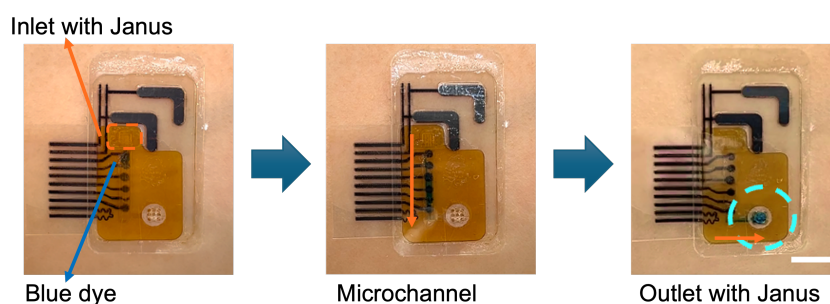


Figure 2.13: Full-system on-body flow test after in vitro transport characterization. Sweat enters through the Janus inlet, and blue dye added near the inlet at the first electrode is carried through the microchannel toward the Janus outlet. Scale bar, 5 mm. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

With that workflow established, we next evaluated whether the same transport capability could still be observed at approximately 24 h after a single carbachol iontophoresis session. Fig. 2.14 shows four representative subjects under this delayed post-induction condition. Because carbachol can sustain sweat induction well be-

yond the initial high-flow period, this experiment evaluates whether improved sweat sampling can maximize the usable lifetime of a single stimulation event, reduce the number of required IP sessions, and extend operation into a lower-flow regime that is especially relevant for practical sensing.

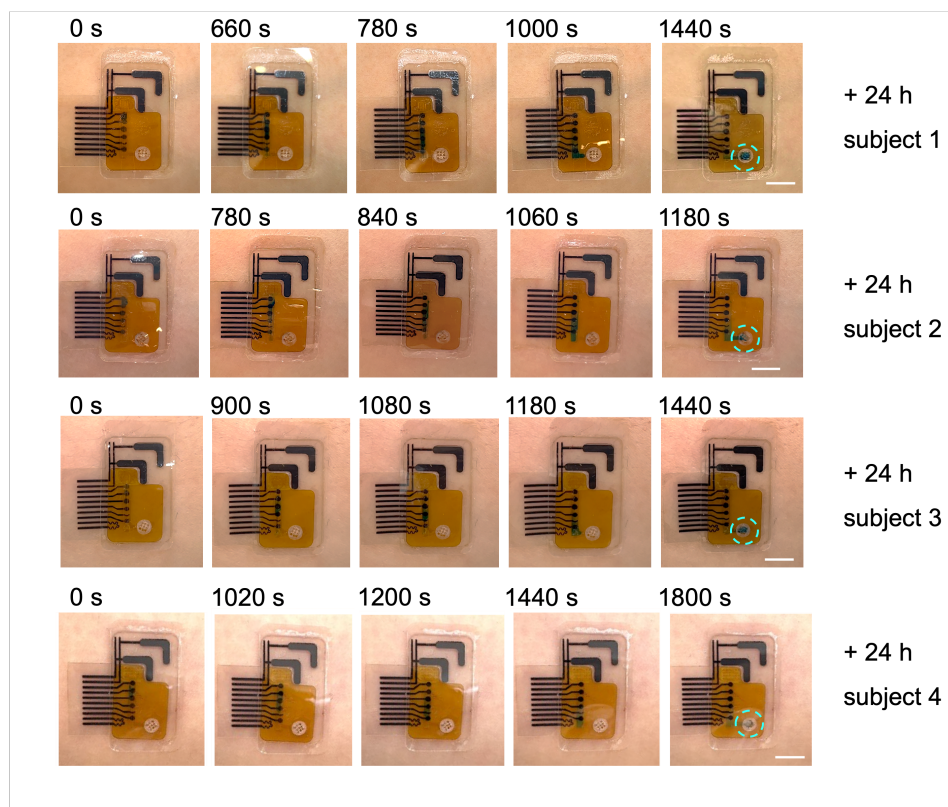


Figure 2.14: On-body transport at approximately 24 h after a single carbachol iontophoresis session. Scale bars, 5 mm. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Fig. 2.14 shows that the integrated patch remains capable of transporting fluid on body at approximately 24 h after stimulation. Reproducibility across multiple subjects indicates that this behavior is not limited to an isolated example. This result shows that the extended lifetime of carbachol-induced sweating can be used more efficiently when the sampling architecture is transport-efficient, thereby reducing the need for repeated iontophoresis while extending operation into a lower-flow sensing regime.

We then asked whether transport could still be observed at an even later time point, where the remaining sweat input approaches a near-passive condition. Fig. 2.15

shows a sequential measurement at 48 h after induction and therefore tests the same system in a much lower-input regime.

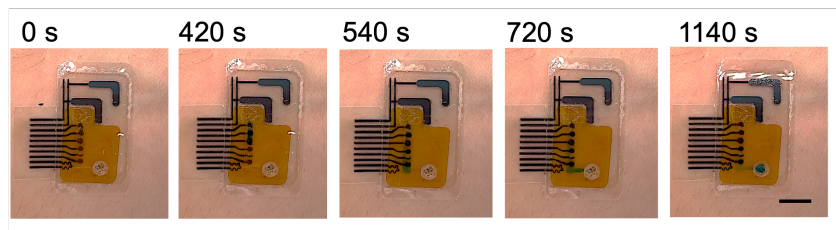


Figure 2.15: On-body transport at 48 h after a single carbachol iontophoresis session. Scale bar, 5 mm. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Fig. 2.15 shows that transport remains observable even at 48 h, although the propagation time is longer than at 24 h. This result indicates that the bioinspired transport architecture remains functional even when fluid availability becomes extremely limited. In practical terms, it further supports the conclusion that the platform can maximize the benefit of a single stimulation event and move wearable sweat sampling closer to passive operation.

2.4 Discussion and Conclusion

This chapter shows that the central challenge in wearable sweat sampling is not simply generating sweat, but transporting small and time-varying amounts of fluid in a reliable way. The results demonstrate that stable operation under low-input conditions depends on coordinated control of fluid capture, channel propagation, and outlet release, rather than on any single design feature alone.

The integrated architecture developed here addresses that requirement at multiple scales. At the inlet, the Janus interface localizes fluid and promotes vertical transfer into the channel. Within the device, the hierarchically structured microchannel enables early transition to continuous flow and sustained propagation. At the outlet, the second Janus element reduces backpressure and stabilizes transport after filling. These elements convert intermittent, accumulation-driven motion into more continuous flow across the full patch, as confirmed by both component-level measurements and whole-device comparisons.

An important outcome of this design is that it extends the usable sampling window after a single iontophoresis session. Because carbachol can sustain secretion for

many hours while the sweat rate gradually declines, practical sampling requires a system that remains functional even when fluid availability becomes very low. The on-body results in this chapter show that the device can still transport sweat at extended times after induction, indicating that prolonged stimulation can be translated into a meaningful sampling window rather than being lost to poor transport efficiency.

These results establish a system-level transport framework for wearable sweat collection under physiologically relevant low-flow conditions. This is important not only for improving sampling efficiency, but also for reducing dependence on repeated stimulation and enabling more practical continuous monitoring. The transport strategy developed here provides the basis for the sensing integration described in the following chapter.

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Chapter 3

SYSTEM INTEGRATION WITH ELECTROCHEMICAL SENSING

Materials from this chapter also appear in “Shin, Soyoung, Ruixiao Liu, Yiran Yang, José A. Lasalde-Ramírez, Gwangmook Kim, Chihyeong Won, Jihong Min, Canran Wang, Kexin Fan, Hong Han, Chibuike Uwakwe, Wenzheng Heng, Tzung K. Hsiai, Zhaoping Li, John D. FitzGerald, and Wei Gao (Aug. 2025). ‘A bioinspired microfluidic wearable sensor for multiday sweat sampling, transport, and metabolic analysis’. In: *Science Advances*.” [1]

The microfluidic platform developed in Chapter 2 establishes a framework for reliable sweat sampling under low secretion conditions by sustaining fluid transport over extended periods. The next step is to integrate sensing elements that can operate within the same low-volume, continuously refreshed environment.

In this chapter, the sensing platform is introduced first at the substrate and electrode-material level, before the addition of mediator, stabilizer, and enzyme layers. This distinction is important because the baseline electrode architecture determines mechanical flexibility, electrical stability, and compatibility with multiplexed functionalization. The chapter then builds from this base structure toward analyte-specific electrochemical sensing within the integrated wearable format.

3.1 Sensor Integration Strategy

The electrochemical sensing platform in this work is designed to enable reliable and multiplexed biochemical analysis within a compact, wearable format, building on prior skin-interfaced sweat-sensor platforms and integrated wearable electrochemical systems [2–5]. The integration strategy begins with a flexible printed electrode array that defines the physical and electrical foundation of the sensing system.

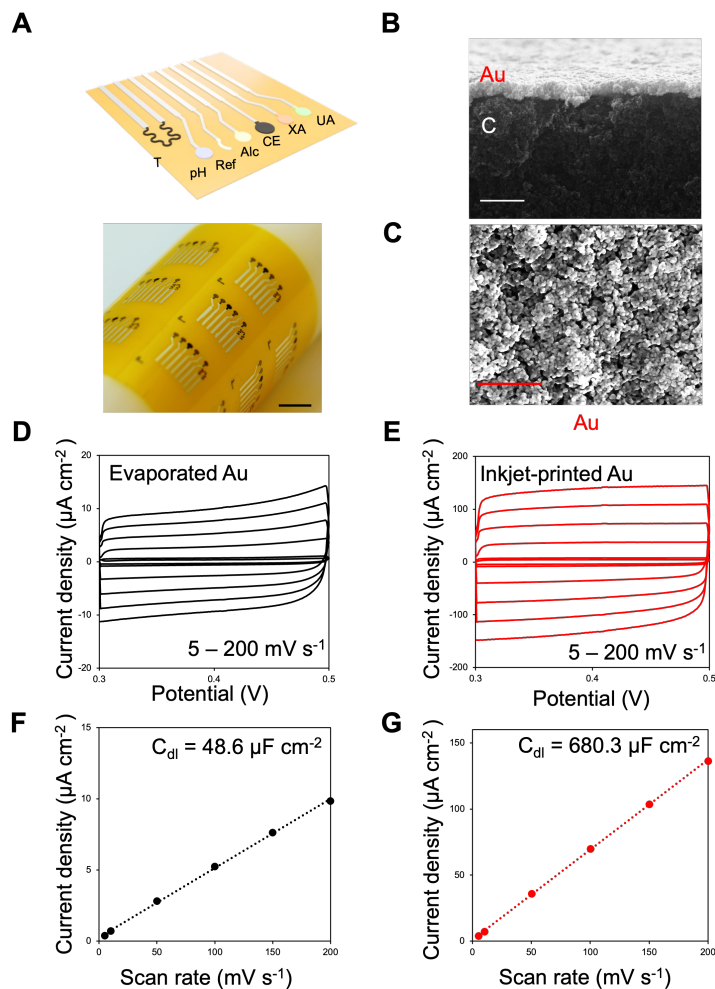


Figure 3.1: Substrate and electrode-material platform for the multiplexed sensing array. (A) Flexible electrode-array layout and printed electrode photograph. (B) Cross-sectional SEM image of Au on carbon. (C) Surface SEM image of the printed Au electrode. (D,E) Cyclic voltammograms of evaporated and inkjet-printed Au electrodes. (F,G) Scan-rate-dependent oxidation current density for evaporated and inkjet-printed Au electrodes. SEM scale bars, 1 μm . Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 3.1 introduces the base sensing platform before analyte-specific modification. In Fig. 3.1A, the schematic and photograph show a compact flexible array containing multiple working electrodes, shared counter and reference electrodes, and auxiliary pH and temperature sites. This layout enables parallel biochemical measurements while keeping the electrochemical environment consistent across channels.

Figures 3.1B–C establish the material basis of the array. The cross-sectional SEM image verifies the Au-on-carbon electrode stack, and the surface SEM image shows the

rough printed Au morphology used for subsequent mediator and enzyme deposition. Figures 3.1D–G compare evaporated and inkjet-printed Au electrodes electrochemically through cyclic voltammetry and scan-rate-dependent current measurements. These structural and electrochemical results show that the printed Au electrodes provide a flexible, active base for the multilayer sensing interface.

The electrode array was designed to combine multiplexed sensing, environmental correction, and portable readout in one platform. Each working electrode can be functionalized with a distinct sensing chemistry, while the shared reference/counter electrodes reduce device complexity. Integrated pH and temperature sensors provide local correction parameters, and compact electronics support multi-channel amperometric and potentiometric acquisition during on-body operation.

3.2 Enzymatic Sensing Platform

The electrochemical sensing platform in this work is built around enzymatic detection, in which biochemical reactions are converted into electrical signals through a mediator-assisted redox process. This strategy is widely used in analytical electrochemistry and wearable biosensing because enzyme specificity can be coupled to amperometric transduction for selective biomarker measurement [4, 6, 7]. It enables selective and sensitive measurement of multiple target analytes within a unified sensing architecture while remaining compatible with continuous wearable operation.

For detection of purine metabolites and alcohol, enzyme-specific reactions are implemented using xanthine oxidase, uricase, and alcohol oxidase, respectively. Prior wearable and electrochemical studies have demonstrated the relevance of sweat uric acid sensing, xanthine sensing, and alcohol monitoring for metabolic and lifestyle-related analysis [8–15]. Each enzyme catalyzes oxidation of its target analyte in the presence of oxygen, generating hydrogen peroxide as a byproduct. The hydrogen peroxide is then detected electrochemically, providing a common transduction pathway across all sensing channels. This shared signal mechanism simplifies multiplexed sensor design while preserving analyte specificity through enzyme selection.

To enable low-potential operation and reduce interference from other electroactive species in sweat, Prussian blue (PB) is used as a redox mediator to catalyze hydrogen peroxide reduction near 0 V.[16–18] Operating near zero potential is especially important in wearable sensing, where complex biofluid compositions can introduce substantial background current. The mediator-based design therefore improves se-

lectivity while supporting stable signal transduction under physiologically relevant conditions.

PB alone, however, has limited operational stability because it is susceptible to degradation, particularly under alkaline conditions.[18, 19] To address this weakness, a nickel hexacyanoferrate (NiHCF) layer is introduced above the PB mediator as a stabilizing component. NiHCF-containing hexacyanoferrate bilayers have been reported to preserve H_2O_2 electrocatalytic activity while substantially improving operational stability compared with PB alone, supporting its use here as a protective and stabilizing overlay.[20] The combined PB–NiHCF architecture thus provides both high sensitivity and improved long-term stability.

The resulting electrode structure and layer formation were characterized using scanning electron microscopy (SEM), which revealed well-defined multilayer architectures composed of the conductive substrate, mediator layer, and stabilizing coating. Cross-sectional and surface images confirmed uniform deposition and consistent morphology of the PB and NiHCF layers across the electrode surface. This controlled interface formation supports reproducible electrochemical behavior and reliable signal generation across sensing sites.

To match analytes with different physiological concentration ranges, the mediator deposition strategy was tailored for each sensing channel. For xanthine and uric acid detection in the micromolar range, analyte flux and enzymatically generated H_2O_2 are relatively limited; therefore, an overly thick PB layer would act as a diffusion barrier and reduce sensitivity. A thin PB layer was therefore deposited using a pulsed electrodeposition method to shorten the diffusion path and maximize interfacial sensitivity. By contrast, alcohol detection occurs in the millimolar range and produces a much larger H_2O_2 signal, creating a greater risk of signal saturation and local mediator depletion. For this channel, a thicker PB layer formed by cyclic voltammetry was used to improve signal stability, serve as a larger mediator reservoir, accommodate the higher current range, and extend dynamic range. This differentiation allows each sensor to operate within its optimal regime while preserving a consistent overall platform design.

Enzyme immobilization was likewise engineered to balance activity, stability, and retention at the electrode interface. Immobilization matrices and diffusion-regulating overlayers are common strategies for preserving enzyme function while controlling substrate access in electrochemical biosensors [6, 16]. For xanthine and uric acid sensors, the enzymes were incorporated into a bovine serum albumin–glutaraldehyde

(BSA–GA) crosslinked network that provides a stable and biocompatible environment. For the alcohol sensor, a composite matrix of chitosan and BSA was used, followed by a polyurethane overlayer to regulate analyte diffusion. These material choices preserve enzymatic function while controlling analyte access and maintaining structural integrity.

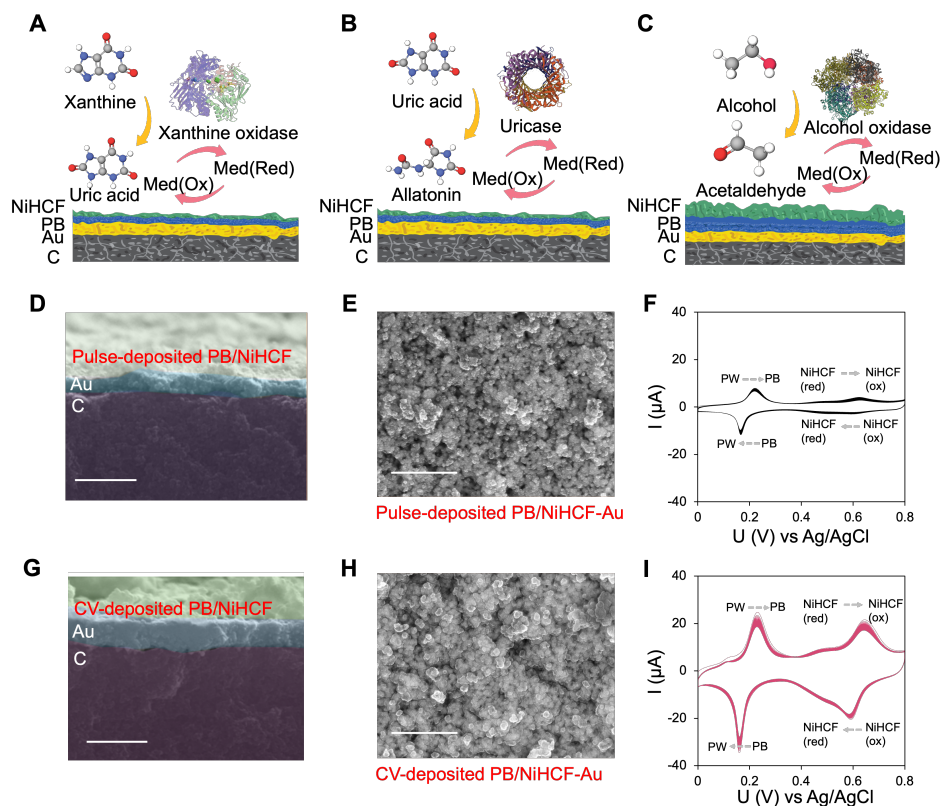


Figure 3.2: Layer modification and electrochemical stabilization of the enzymatic sensing interface. (A–C) Schematic sensing architectures for xanthine, uric acid, and alcohol. (D–F) Pulse-deposited PB/NiHCF layer for the xanthine and uric acid sensors: cross-sectional SEM, surface SEM, and cyclic voltammetry. (G–I) CV-deposited PB/NiHCF layer for the alcohol sensor: cross-sectional SEM, surface SEM, and cyclic voltammetry. Scan rate, 50 mV s^{-1} . SEM scale bars, $1 \mu\text{m}$. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 3.2 presents the sensing interface from reaction design to layer characterization. In Fig. 3.2A–C, xanthine, uric acid, and alcohol use different enzymes but share PB/NiHCF-mediated H_2O_2 reduction as the electrochemical readout. Biochemical selectivity is therefore set by the enzyme layer, while the mediator layer provides a common low-potential transduction pathway. Figures 3.2D–F characterize the pulse-

deposited PB/NiHCF layer used for xanthine and uric acid sensing, and Figs. 3.2G–I show the CV-deposited layer used for alcohol. The SEM and cyclic-voltammetry data confirm continuous coating, rough mediator morphology, and retained PW/PB and NiHCF redox features for both deposition strategies.

The enzymatic sensors were first evaluated through calibration and long-term operation to determine whether the PB/NiHCF mediator–stabilizer architecture supported both quantitative sensitivity and sustained readout. As shown in Fig. 3.3, the uric acid, xanthine, and alcohol channels exhibit concentration-dependent amperometric responses with linear calibration behavior across their target ranges. This confirms that enzymatically generated H_2O_2 is transduced effectively through the mediator layer and that the deposition strategies preserve useful sensitivity for both micromolar and millimolar analytes. The long-term trace shows stable baselines, clear responses to concentration changes, and continued operation over prolonged measurement, supporting the use of the mediator and stabilizing layers for continuous monitoring.

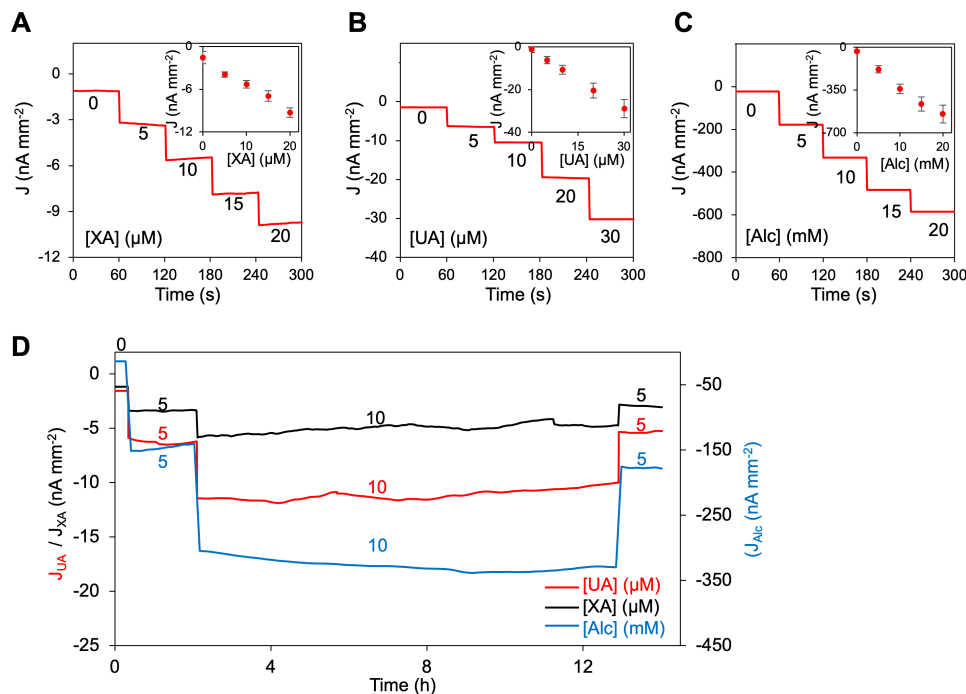


Figure 3.3: Calibration and long-term operation of the multiplexed enzymatic sensors. (A–C) Representative amperometric calibration responses and linear calibration plots for the uric acid, xanthine, and alcohol sensors. (D) Long-term operation under stepwise analyte concentrations. Error bars indicate s.d. where applicable. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Because the system relies on carbachol-induced sweat for extended sampling, calibration should reflect the pH encountered during induction rather than an idealized neutral condition. Figure 3.4A shows that carbachol-induced sweat is centered near pH 9, so pH 9 was selected for enzymatic sensor evaluation. This condition is important because alkaline environments can destabilize PB-based electrochemistry or alter enzyme activity. Figures 3.4B–D compare sensor readout with independent assay-kit measurements, showing that the selected calibration condition still produces quantitatively meaningful analyte measurements.

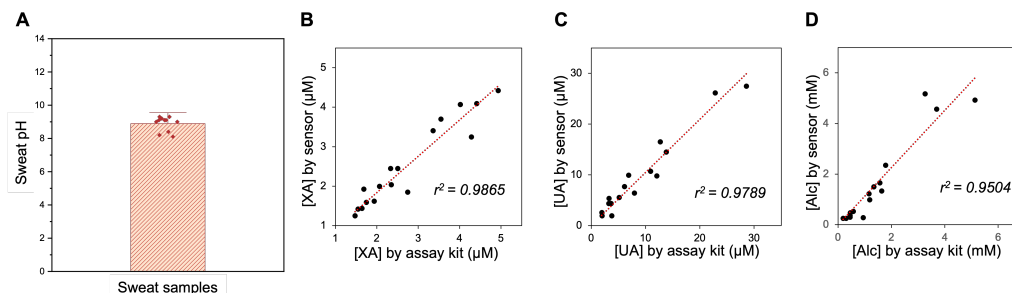


Figure 3.4: Sweat pH and assay validation under the selected calibration condition. (A) pH of carbachol-induced sweat samples. (B–D) Comparison of sensor readout with assay-kit measurements for the target analytes. Error bars indicate s.d. where applicable. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

After calibration and assay validation, the sensors were evaluated for reversibility, selectivity, and shelf stability. Figure 3.5 organizes these results by analyte: xanthine in Figs. 3.5A–B, uric acid in Figs. 3.5C–D, and alcohol in Figs. 3.5E–F. The reversibility/selectivity panels test whether the sensors follow increasing and decreasing analyte concentrations while rejecting common sweat interferents. The shelf-stability panels show whether useful signal contrast is retained after storage. These measurements extend the calibration results by showing that the sensors remain reversible, selective, and storage-stable.

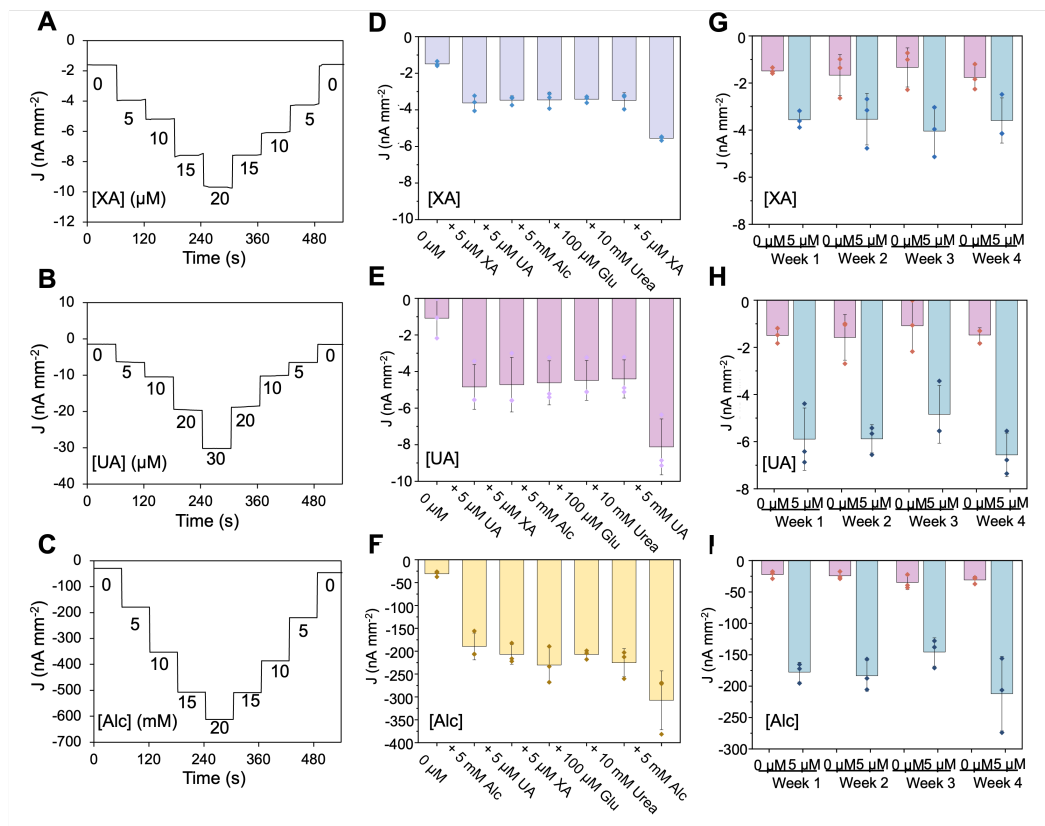


Figure 3.5: Reversibility, selectivity, and shelf stability of the enzymatic biosensors. (A,B) Xanthine sensor reversibility/selectivity and shelf stability. (C,D) Uric acid sensor reversibility/selectivity and shelf stability. (E,F) Alcohol sensor reversibility/selectivity and shelf stability. Error bars represent s.d. of the mean from 3 sensors. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Long-term signal retention does not by itself guarantee accurate sensing during wearable operation. Enzyme activity, mediator behavior, and reaction kinetics can shift with local pH and temperature, so biochemical currents must be interpreted together with environmental measurements rather than under a fixed nominal calibration state. This need for local correction is consistent with broader wearable sweat-sensing systems that integrate pH, temperature, ionic-strength, or related auxiliary measurements to calibrate biochemical readouts [2, 21–24]. The pH sensor itself was therefore characterized structurally and electrochemically, as shown in Fig. 3.6A–D: the SEM images in Fig. 3.6A–B verify the PANI coating morphology in both surface and cross-sectional views, Fig. 3.6C confirms repeated PANI electrodeposition over 12 cycles at 100 mV s^{-1} , and Fig. 3.6D demonstrates reversible potentiometric operation during pH cycling from 9 to 4 and back to 9.

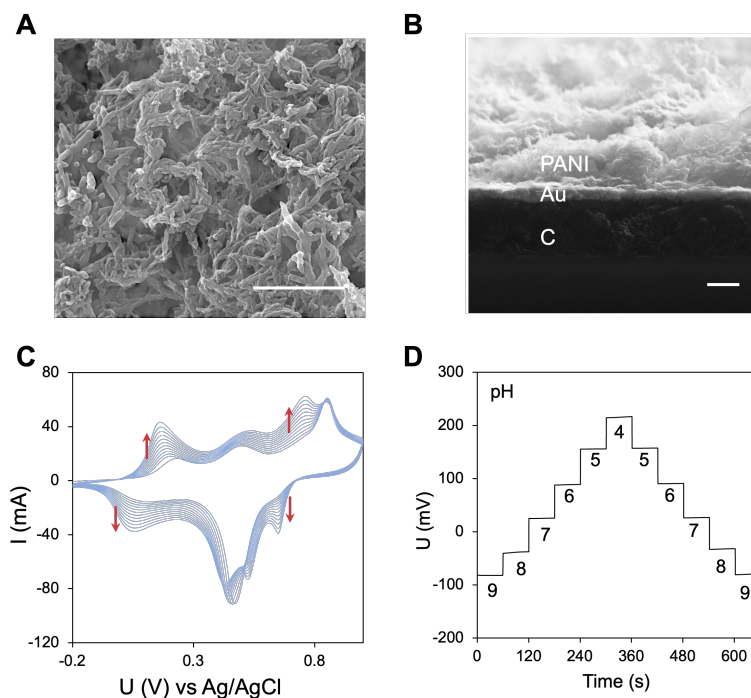


Figure 3.6: PANI-based pH sensor characterization. (A,B) Surface and cross-sectional SEM images of the PANI-coated electrode. (C) Cyclic-voltammetry traces during repeated PANI electrodeposition over 12 cycles at 100 mV s^{-1} . (D) Reversible potentiometric pH response from pH 9 to pH 4 and back to pH 9. Scale bars, $1 \mu\text{m}$ for SEM images in (A) and (B). Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

This pH-sensor characterization supports the use of the auxiliary pH channel as an active correction input rather than a purely descriptive measurement. By confirming coating morphology, electrodeposition behavior, and reversible potentiometric response, Fig. 3.6 establishes that local pH can be tracked alongside the enzymatic metabolite channels during multiplexed operation.

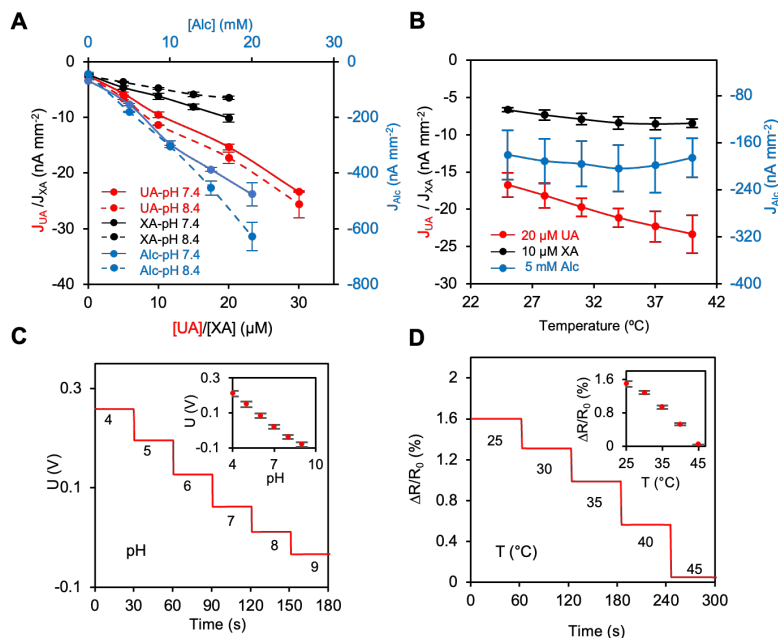


Figure 3.7: Need for pH and temperature correction and calibration of auxiliary sensors. (A) Effect of pH on uric acid, xanthine, and alcohol sensor responses. (B) Effect of temperature on sensor responses. (C) pH sensor response and calibration. (D) Temperature sensor response and calibration. Error bars indicate s.d. where applicable. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 3.7A–D shows how pH and temperature affect the biochemical channels and how the auxiliary sensors provide correction inputs. In Figs. 3.7A–B, the uric acid, xanthine, and alcohol responses vary with acid–base and thermal conditions, indicating that current changes could be misread as concentration changes without environmental context. Figures 3.7C–D show monotonic pH and temperature sensor responses that can be used to support correction during operation.

The multiplexed configuration enables simultaneous tracking of metabolites that are linked through purine metabolism and alcohol intake. Measuring xanthine, uric acid, and alcohol together provides a broader biochemical context than single-analyte sensing, while integrated pH and temperature channels support interpretation of those signals during changing on-body conditions. This approach follows the broader trend toward multimodal wearable systems that combine biochemical, physiological, and environmental signals for more reliable interpretation [3, 5, 25–28].

Accurate multiplexed sensing also requires attention to interactions between channels. One practical issue is signal scale: alcohol sensing produces microampere-

level currents, whereas xanthine and uric acid operate in the nanoampere range. The readout framework therefore needs sufficient dynamic range to capture high-current alcohol signals without losing resolution for lower-current purine metabolites.

Biochemical coupling between analytes is another consideration. Xanthine detection generates uric acid as a reaction product, which could artificially elevate the uric-acid signal if the two channels are not interpreted carefully. The sensing configuration therefore treats uric acid independently from xanthine-derived products so that the uric-acid channel reflects the native concentration in the sampled biofluid. Accounting for both signal scale and biochemical coupling helps make the multiplexed measurements more interpretable.

These benchtop results establish the sensing interface under controlled conditions. Chapter 4 next examines whether the same sensing performance can be maintained when the electrodes are coupled to microfluidic transport and evaluated under on-body operating conditions.

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Chapter 4

REAL-WORLD AND CLINICAL VALIDATION OF ON-BODY SWEAT MONITORING

Materials from this chapter also appear in “Shin, Soyoung, Ruixiao Liu, Yiran Yang, José A. Lasalde-Ramírez, Gwangmook Kim, Chihyeong Won, Jihong Min, Canran Wang, Kexin Fan, Hong Han, Chibuike Uwakwe, Wenzheng Heng, Tzung K. Hsiai, Zhaoping Li, John D. FitzGerald, and Wei Gao (Aug. 2025). ‘A bioinspired microfluidic wearable sensor for multiday sweat sampling, transport, and metabolic analysis’. In: *Science Advances*.” [1]

A central objective of this chapter is to move sweat sensing beyond controlled laboratory demonstrations toward realistic on-body and clinically relevant use. Although benchtop characterization establishes sensor sensitivity and material stability, practical wearable monitoring also requires evidence that the integrated system can operate on skin, over extended time windows, and under conditions where sweat secretion, activity, diet, and medication use vary. This chapter addresses that gap by evaluating the full BMS³ platform in real-world and clinical contexts, including ultra-low-flow sensing, portable PCB-based readout, multi-day on-body monitoring, and gout-related allopurinol studies.

4.1 System Validation and Wearable Implementation

The integrated system was first validated at the device level to confirm that both the sensing interface and microfluidic architecture maintain performance after fabrication and system assembly. The wearable platform consists of stacked functional layers, including the sensing electrodes, microfluidic transport layers, and Janus interfaces, forming a fully integrated patch. This layered design enables seamless coupling between fluid transport and electrochemical detection. The complete fabrication and assembly workflow is shown in Fig. 4.1.

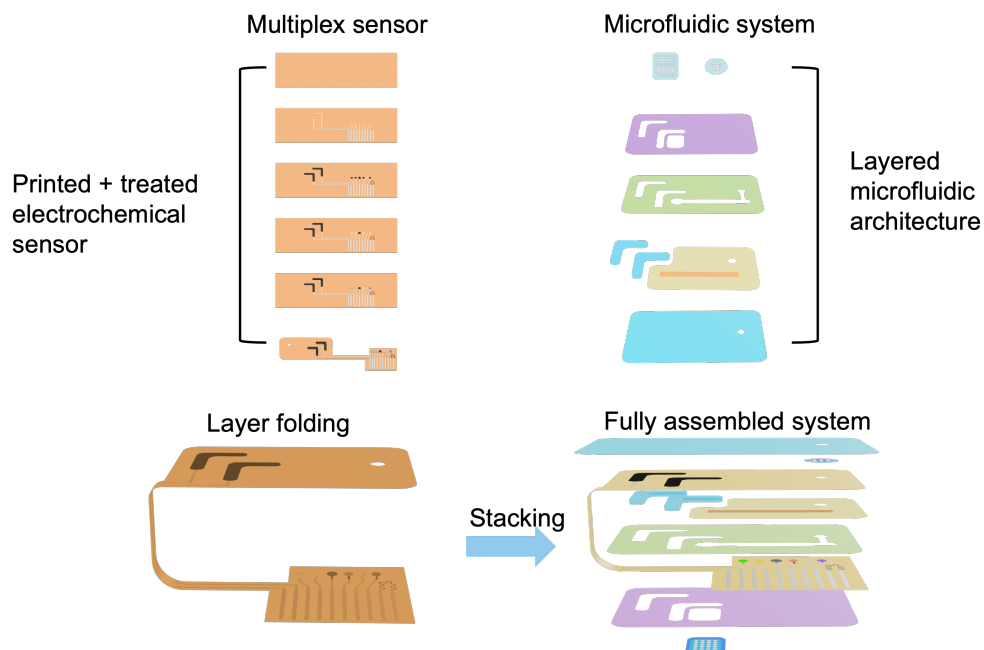


Figure 4.1: Fabrication and assembly workflow for the integrated BMS³ platform. The schematic summarizes preparation of the flexible sensing array, patterned transport components, Janus inlet/outlet interfaces, and final multilayer assembly used to couple sweat collection, microfluidic transport, and electrochemical sensing. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.1 is included in the main chapter because the assembly sequence is central to interpreting the validation results. The performance of the platform depends on alignment between the sweat-collection interface, transport layers, and electrochemical sensing array; therefore, the fabrication workflow directly explains how the device architecture enables the low-flow experiments and on-body studies that follow.

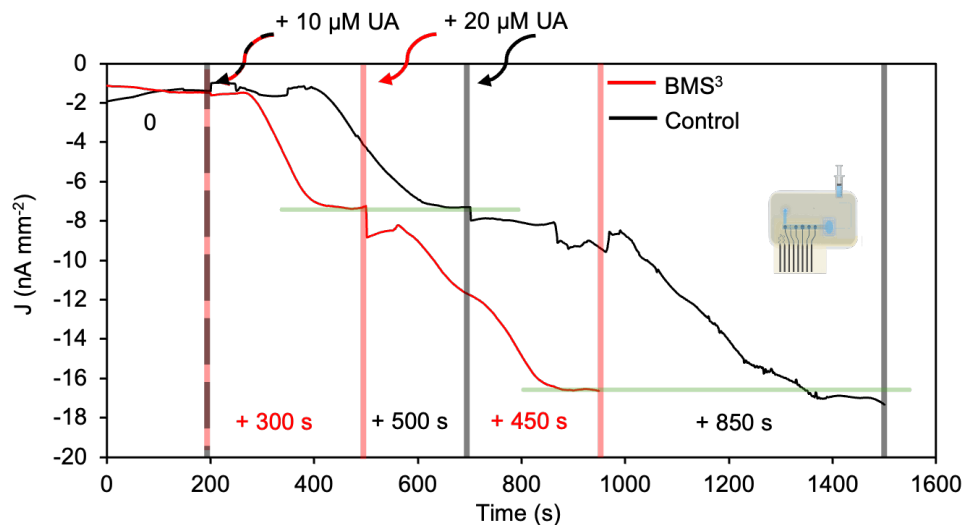


Figure 4.2: Comparison of uric-acid sensing response with and without the integrated transport system under ultra-low flow conditions ($0.1 \mu\text{L min}^{-1}$). The integrated BMS³ platform (red) is compared with a control configuration without the full transport system (black), with step additions of uric acid indicated on the trace. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.2 provides the first direct evidence in Chapter 4 that sensing remains possible even under an extremely low flow rate of $0.1 \mu\text{L min}^{-1}$. This condition is important because it approaches the regime in which conventional wearable sweat sensors often fail to deliver fresh analyte to the sensing interface quickly enough for practical monitoring. The comparison shows that both configurations can eventually respond to the uric-acid steps, confirming that electrochemical detection is still possible at very low flow. However, the integrated BMS³ platform responds much more rapidly than the control, reaching the new concentration levels hundreds of seconds earlier at both analyte steps. Scientifically, this shows that the transport architecture does not merely preserve sensing at low flow, but actively improves the refreshment of analyte at the sensor surface, thereby reducing lag and making low-flow continuous monitoring more realistic.

To further interpret sensing behavior under continuous low-flow operation, several dimensionless transport parameters were considered to evaluate the relative contributions of convection, diffusion, and enzymatic reaction kinetics within the system. These analyses provide physical insight into how analyte transport and reaction dynamics influence sensor response during wearable operation, following standard

descriptions of transport phenomena, microfluidic flow, and electrochemical sensing systems [2–4].

The Reynolds number (Re), which describes the ratio of inertial to viscous forces in fluid flow, is expressed as:

$$Re = \frac{\rho U D_h}{\mu} \quad (4.1)$$

where ρ is fluid density, U is average flow velocity, D_h is hydraulic diameter, and μ is dynamic viscosity. Under the operating conditions used in this system, the calculated Reynolds number remains significantly below unity ($Re \ll 1$), indicating fully laminar flow within the microchannel. This behavior is expected for low-volume sweat transport, where viscous forces dominate fluid motion.

To evaluate transport along the microchannel, the Péclet number (Pe) was considered:

$$Pe = \frac{UL}{D} \quad (4.2)$$

where L is the characteristic transport length and D is the analyte diffusion coefficient. The calculated Péclet numbers for alcohol, uric acid, and xanthine are substantially greater than unity ($Pe \gg 1$), indicating that transport along the channel is governed primarily by advection rather than diffusion. This result highlights the importance of continuous fluid refreshment at the sensing interface, as analyte replenishment depends strongly on sustained flow through the microfluidic system.

The balance between analyte delivery and enzymatic conversion was further evaluated using the Damköhler number (Da):

$$Da \sim \frac{V_{\max}}{QC} \quad (4.3)$$

where $V_{\max}$ is the maximum enzymatic reaction rate, Q is volumetric flow rate, and C is analyte concentration. The estimated Damköhler numbers are substantially greater than unity ($Da \gg 1$), suggesting that enzymatic conversion occurs more rapidly than analyte delivery to the sensing region. These results indicate that sensor response is primarily governed by mass transport rather than intrinsic enzyme kinetics.

In addition to transport along the channel, vertical diffusion through the transport and enzyme layers contributes to the overall response time of the sensing system. The characteristic diffusion timescale can be approximated as:

$$t \sim \frac{L^2}{D} \quad (4.4)$$

where L represents the diffusion distance through the corresponding layer. Based on the estimated layer thicknesses used in this platform, diffusion across the enzyme layer occurs over relatively short timescales, whereas transport through the full adhesive transport layer occurs more slowly. These estimates suggest that vertical diffusion contributes to response delay, while continuous lateral flow remains necessary to maintain analyte renewal during operation.

These transport analyses support that sensing behavior within the platform is governed primarily by controlled analyte delivery and sustained refreshment under low-flow conditions. These considerations further emphasize the importance of integrating electrochemical sensing with continuous microfluidic transport in wearable sweat sensing systems.

Detailed structural and benchtop electrochemical characterization of the sensing electrodes was presented in Chapter 3 and is not repeated here. Those earlier results established that the multilayer sensing interfaces were stable, selective, and sufficiently robust for integrated operation. Chapter 4 therefore focuses on the next system-level question: whether those validated sensing elements retain their performance once incorporated into the wearable platform and portable electronics.

To address this, the system was coupled to a custom printed circuit board (PCB) for portable data acquisition. Figure 4.3 shows that, after PCB integration, the platform still preserves clear concentration-dependent responses across all sensing channels, including xanthine, uric acid, alcohol, pH, and temperature. In other words, the portable readout does not obscure the underlying electrochemical behavior of the patch, but instead enables that behavior to be measured in a format suitable for on-body studies.

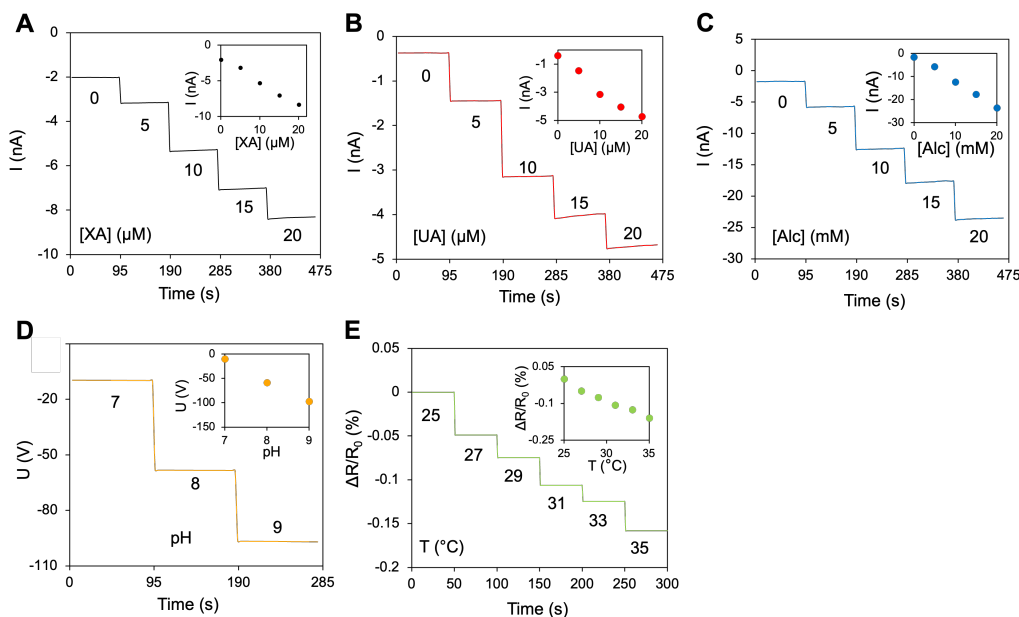


Figure 4.3: Calibration of microfluidic BMS³ sensor arrays with PCB. (A–E) The PCB-recorded electrochemical response of xanthine (A), uric acid (B), alcohol (C), pH (D), and temperature (E) sensors in the microfluidics. Detection was performed under a controlled flow rate of $0.2 \mu\text{L min}^{-1}$. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figures 4.3A–C show that the three biochemical sensing channels retain clear step-wise responses and concentration-dependent calibration behavior after PCB integration. This is important because the portable readout must preserve both sensitivity and dynamic range rather than merely recording noisy current traces. Figures 4.3D–E then show the corresponding pH and temperature channels, confirming that the auxiliary sensors used for environmental correction can also be read reliably through the same hardware interface. The purpose of including the PCB here is therefore not to analyze the circuit in detail, but to show that the fully integrated sensing platform remains measurable in a portable format before moving to the on-body experiments.

Taken together, this validation shows that the fully integrated platform retains sensing accuracy and stability beyond controlled electrochemical testing. It therefore provides a reliable foundation for subsequent on-body studies, where the system operates under physiologically relevant conditions and low sweat rates.

4.2 On-Body Evaluation Under Physiological Conditions

This section evaluates system performance under on-body conditions, where sweat secretion and metabolic signals vary with time, activity, and stimulation method. The focus is to understand how time-dependent sweat rate dynamics define the effective sensing window and how the system operates under realistic physiological conditions.

Before presenting the on-body datasets, it is important to clarify why intake studies, activity-dependent measurements, and continuous monitoring are particularly relevant for the biomarkers examined here. Xanthine and uric acid are not independent readouts, but linked components of purine metabolism whose relative behavior can shift with food intake, alcohol exposure, physical activity, oxidative stress, and pharmacological inhibition. In this pathway, xanthine oxidase converts xanthine to uric acid, whereas allopurinol and its metabolite oxypurinol suppress this enzymatic step. Alcohol intake can further perturb the pathway by increasing purine turnover through ATP degradation, thereby coupling behavioral inputs to downstream uric-acid production. For this reason, single time-point measurements provide only limited information: distinguishing transient intake- or activity-driven fluctuations from sustained metabolic changes requires continuous monitoring over time.

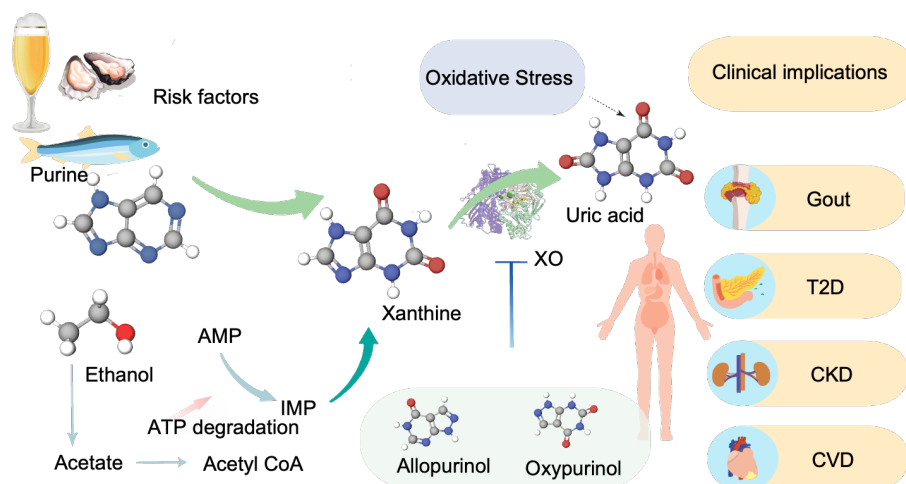


Figure 4.4: Mechanistic illustration of purine metabolism and its clinical implications. High-purine diets and alcohol consumption elevate circulating purine metabolites, including xanthine and uric acid, contributing to oxidative stress and increasing the risk of disorders such as gout, type 2 diabetes (T2D), chronic kidney disease (CKD), and cardiovascular disease (CVD). Xanthine oxidase (XO) catalyzes the conversion of xanthine to uric acid, whereas allopurinol and oxypurinol inhibit this enzymatic step. In parallel, alcohol metabolism promotes ATP degradation pathways that increase purine turnover and ultimately contribute to uric-acid generation. This pathway-level framework motivates the intake, activity, and pharmacological studies presented in this section. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.4 therefore provides the physiological rationale for the on-body experiments that follow. Because diet, alcohol exposure, and daily activity can perturb purine metabolism on overlapping but different timescales, wearable monitoring is most informative when the sensing system can follow these changes continuously rather than only at isolated sampling points. The on-body studies in this section are designed with that goal in mind: they test whether the integrated platform can track pathway-relevant biochemical fluctuations while operating within the practical constraints of sweat generation and low-flow transport.

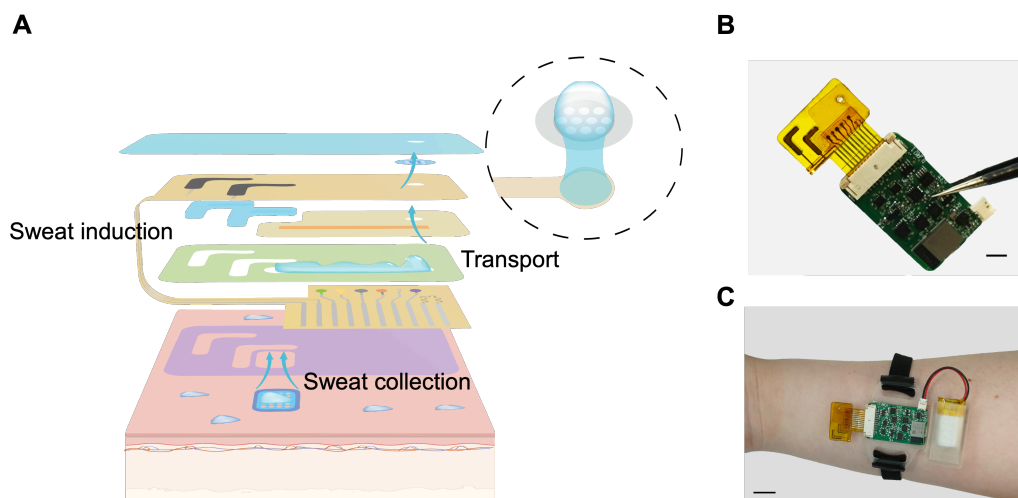


Figure 4.5: Overview of the fully integrated on-body sensing platform. (A) Exploded schematic of the wearable patch showing the stacked functional layers used for sweat collection, microfluidic transport, and electrochemical sensing. (B) Photograph of the integrated sensing patch connected to the portable PCB-based readout hardware. (C) Representative on-body image of the fully assembled system during wear. Scale bars, 5 mm in (B) and 1 cm in (C). Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.5 introduces the fully integrated platform used for the on-body studies. Figure 4.5A clarifies how the sensing electrodes, transport layers, and sweat-collection interface are physically combined into a single stacked architecture, which is important because the on-body measurements in this chapter depend on the coordinated function of all of these layers rather than on the sensing electrode alone. Figures 4.5B–C then show the realized system in hardware form and during wear. The portable PCB is included because it enables signal acquisition from the integrated patch, but it is not treated here as a subject of detailed engineering analysis. Instead, its role in this chapter is simply to support portable on-body readout so that the focus remains on whether the integrated microfluidic–electrochemical platform can operate under realistic physiological conditions.

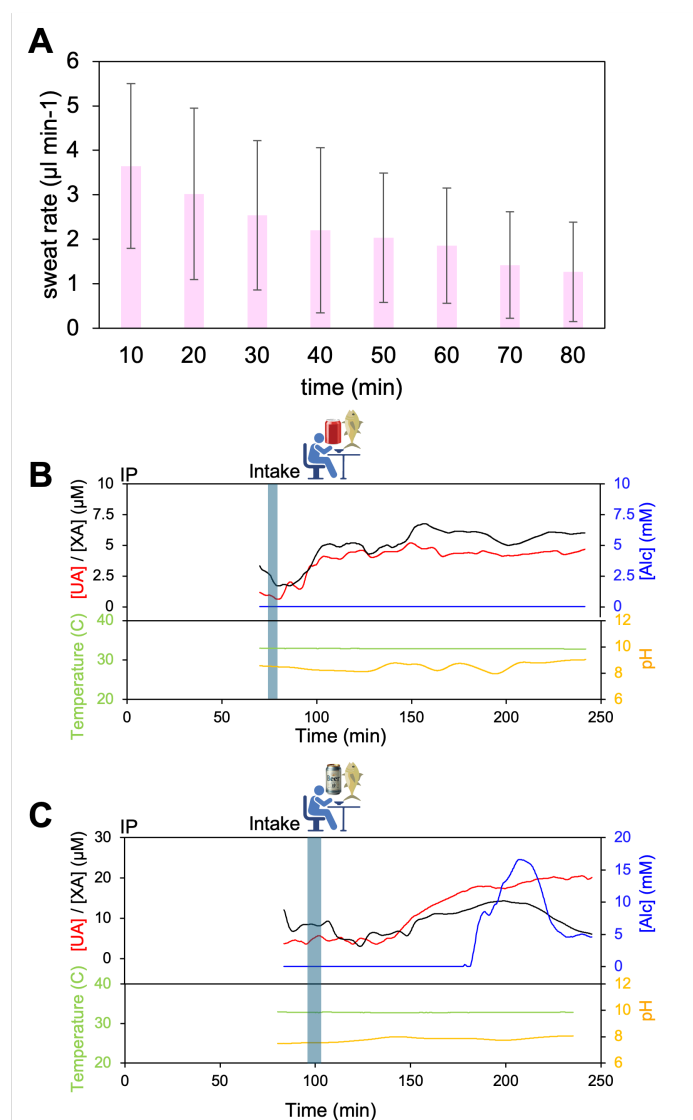


Figure 4.6: On-body evaluation under delayed post-iontophoresis conditions. (A) Average sweat-rate decay measured from 10 subjects after iontophoresis. (B–C) Representative continuous on-body traces collected approximately 1 hour after stimulation during intake-related studies, showing uric-acid and xanthine-related responses together with pH and temperature readouts. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Following iontophoresis using the integrated stimulation module, sweat secretion exhibits a time-dependent decay, with higher initial flow rates that gradually decrease over time. The on-body evaluation in Fig. 4.6A summarizes the averaged sweat-rate behavior from 10 different subjects and shows that the flow can become quite low within less than 1 hour after stimulation. This regime is especially challenging

for wearable electrochemical sensing because limited analyte delivery can increase response lag and make continuous readout less reliable. For this reason, the first on-body tests were intentionally performed about 1 hour after the iontophoresis session, when the system is already operating under constrained fluid availability. This creates a more stringent evaluation of whether the integrated microfluidic transport architecture can still sustain sensing after the initial high-flow period has passed.

Figures 4.6B–C then present representative delayed post-iontophoresis intake studies. These experiments were designed to test whether continuous monitoring can capture pathway-level changes after behavioral perturbation, rather than only isolated concentration snapshots. Following high-purine food and alcohol intake, both uric acid and xanthine exhibit time-dependent changes, consistent with the coupled purine-metabolism framework introduced in Fig. 4.4. The ability to monitor both signals approximately 1 hour after stimulation is important because it shows that the platform remains functional even when sweat supply is already reduced, thereby directly addressing the sensing limitations imposed by low flow.

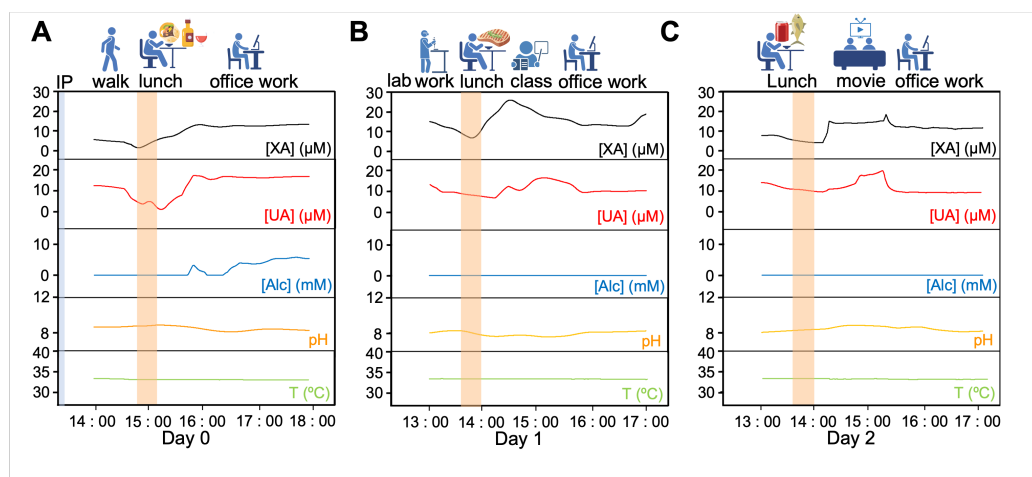


Figure 4.7: Second on-body test set from a single subject across multiple days. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

A second set of on-body experiments was then performed to examine day-to-day reproducibility in a single subject under different behavioral conditions. Figure 4.7 compiles measurements collected across multiple days, with each monitoring session initiated by a single iontophoresis event and followed by distinct patterns of activity. Rather than focusing on one specific perturbation alone, this dataset was intended to

test whether the system can remain operational across repeated real-world sessions that include different combinations of walking, dietary intake, alcohol exposure, sedentary work, and rest.

The multi-day traces show that the platform can continuously follow uric-acid and xanthine dynamics while simultaneously recording pH and temperature across these distinct daily contexts. Importantly, the signal patterns are not identical from day to day, which is expected because purine metabolism depends on the timing and type of intake as well as the surrounding activity level. What remains consistent, however, is the ability of the integrated platform to maintain continuous readout from the same subject over repeated sessions after only a single iontophoresis stimulation per experiment. This result extends the first on-body test set by showing that the system is not limited to one isolated intake event, but can support longitudinal monitoring across multiple days and activity conditions.

Beyond activity-based on-body validation, these results motivate a more clinically focused application in gout management. Gout is driven by chronic hyperuricemia and remains a common inflammatory arthritis with substantial treatment gaps, including incomplete use and adherence to urate-lowering therapy [5, 6]. The biochemical response to therapy is not always obvious to patients on a day-to-day basis. In routine practice, allopurinol is commonly started at a low dose, often 100 mg day^{-1} , and then titrated over time toward urate-lowering goals using intermittent laboratory follow-up [7]. As a result, neither the patient nor the clinician can directly observe whether a given dose is already altering purine-metabolism dynamics in real time. Continuous monitoring could therefore provide additional value by revealing how uric acid and upstream xanthine respond after treatment initiation or dose adjustment, rather than relying only on sparse measurements or delayed symptom relief.

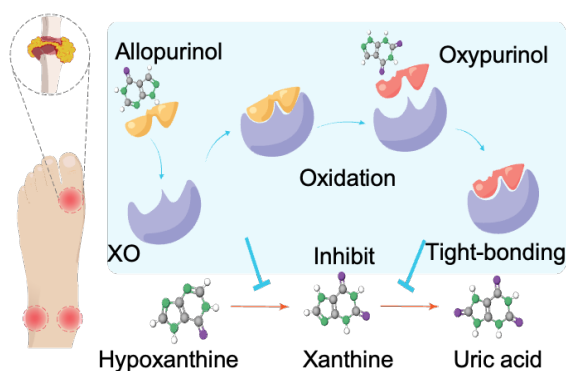


Figure 4.8: Mechanistic schematic of allopurinol action in gout therapy. Allopurinol acts as a structural analog of hypoxanthine and is oxidized by xanthine oxidase to oxypurinol. Both species inhibit xanthine oxidase, but oxypurinol binds more tightly and provides the dominant sustained inhibition, thereby prolonging suppression of xanthine-to-uric-acid conversion [8–10]. This mechanism explains why changes in xanthine and uric-acid levels may evolve over time after dosing and provides a pathway-level rationale for continuous monitoring during gout management. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.8 highlights the pharmacological basis for this application. Allopurinol inhibits xanthine oxidase and is converted to oxypurinol, the main active metabolite responsible for sustained pathway inhibition [11, 12]. Allopurinol itself is relatively short-lived, with an approximate half-life of 1–2 hours, whereas oxypurinol has a much longer half-life of about 18–30 hours [8–10, 13]. Consequently, the biochemical effect of treatment is not determined only by the moment of drug intake, but also by oxypurinol formation and persistence. In the wearable readout used here, effective xanthine-oxidase inhibition is expected to appear as decreased uric-acid output together with a decreased xanthine-related signal after dosing, as evaluated in the patient studies below.

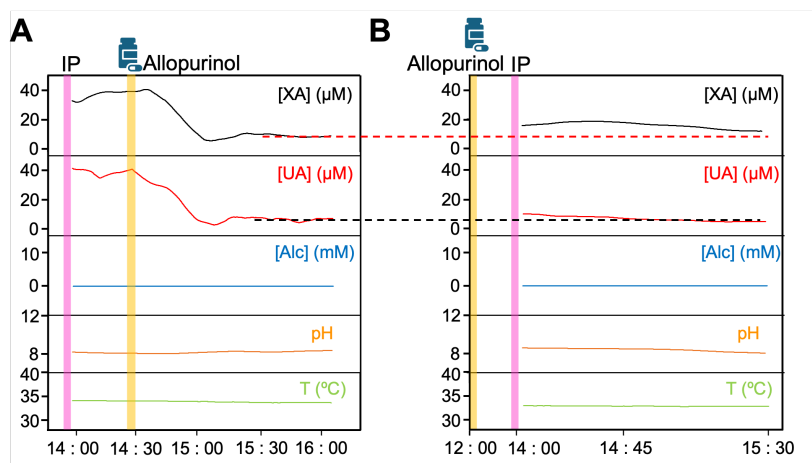


Figure 4.9: Continuous monitoring results from gout-patient studies during pre-scribed allopurinol intake. (A–B) Two different patients with gout were monitored while taking their clinically prescribed allopurinol doses. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

Figure 4.9 extends the gout discussion from pharmacological rationale to patient-level monitoring. Panels A and B show two gout patients taking their prescribed allopurinol regimens, with both uric-acid and xanthine-related signals changing after dosing. In particular, the xanthine channel decreases strongly in both patients, which is consistent with effective suppression of xanthine oxidase by allopurinol and, more importantly, by oxypurinol after metabolic conversion [8–10]. These results illustrate why continuous monitoring can be beneficial in gout: rather than waiting for delayed symptom feedback or intermittent blood testing, the biochemical effect of the prescribed dose can be followed dynamically after administration.

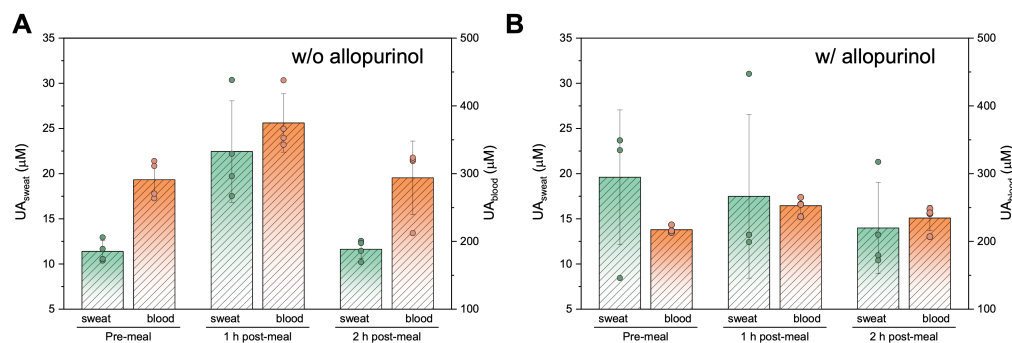


Figure 4.10: On-body evaluation of sweat and blood correlation for purine metabolites. Comparison of uric-acid and xanthine levels measured in sweat and blood for healthy individuals and patients with gout before and after allopurinol intake. Adapted from Ref. [1] under the terms of the CC-BY license. Copyright 2025 The Authors, published by AAAS.

As a final validation step in Chapter 4, sweat measurements were compared with corresponding blood data. Figure 4.10 is included here to show that the broad biochemical trends observed noninvasively are consistent with conventional blood-based measurements. Consistent with the clinical-validation description in the original paper, patients with gout exhibit elevated uric-acid and xanthine levels relative to healthy individuals, and both markers decrease after allopurinol intake. This agreement is important because it supports that the wearable platform is capturing physiologically meaningful changes rather than arbitrary sensor fluctuations.

At the same time, these blood-correlation data should be interpreted in light of the clinical context. The gout patients in this dataset were taking allopurinol routinely, and they reported varying their daily dose depending on circumstances such as diet or alcohol intake. The figure is therefore most valuable as a validation of trend-level correspondence between sweat and blood, rather than as a strictly standardized pharmacokinetic comparison across identical dosing conditions.

Overall, these results demonstrate that system performance is governed not only by sensor characteristics but also by physiological constraints such as sweat rate decay, activity-dependent variability, stimulation conditions, and ultimately the pharmacological context in which the sensors may be used. The ability to sustain transport and sensing under low-flow regimes highlights the importance of system-level design for enabling practical on-body monitoring.

4.3 Summary and System-Level Implications

This chapter demonstrates that the performance of wearable sweat sensing systems is governed not only by sensor characteristics but also by physiological constraints, particularly the time-dependent decay in sweat secretion following iontophoresis. This behavior defines a limited effective sensing window and highlights the importance of evaluating system performance under realistic on-body conditions.

The integrated platform presented in this work enables reliable operation within this constrained regime by combining localized sweat induction with a transport-efficient microfluidic architecture. By sustaining fluid transport under low secretion conditions, the system remains functional beyond peak sweat generation and captures dynamic biochemical changes during daily activities and physiological perturbations.

These results highlight the importance of system-level design in wearable biosensing, where efficient sampling and transport are critical for maintaining signal relevance over time. The ability to operate under low-flow conditions and reduced stimulation requirements provides a pathway toward more practical and continuous on-body monitoring.

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Chapter 5

SUMMARY AND OUTLOOK

Materials from this chapter also appear in “Min, Jihong, Jiaobing Tu, Changhao Xu, Heather Lukas, Soyoung Shin, Yiran Yang, Samuel A. Solomon, Daniel Mukasa, and Wei Gao (Mar. 2023). ‘Skin-Interfaced Wearable Sweat Sensors for Precision Medicine’. In: *Chemical Reviews* 123, pp. 5049–5138. doi: 10.1021/acs.chemrev.2c00823”, “Shin, Soyoung, Ruixiao Liu, Yiran Yang, José A. Lasalde-Ramírez, Gwangmook Kim, Chihyeong Won, Jihong Min, Canran Wang, Kexin Fan, Hong Han, Chibuikwe Uwakwe, Wenzheng Heng, Tzung K. Hsiai, Zhaoping Li, John D. FitzGerald, and Wei Gao (Aug. 2025). ‘A bioinspired microfluidic wearable sensor for multiday sweat sampling, transport, and metabolic analysis’. In: *Science Advances*”, and “Shin, Soyoung, and Wei Gao (May 2026). ‘Making Sweat Measurable: Induction, Sampling, and Refreshment in Wearable Biofluid Sensing’. In: *Advanced Science*. doi: 10.1002/adv.75684.” [1–3]

This thesis developed a bioinspired microfluidic wearable sensing platform for continuous sweat sampling and metabolic monitoring. The central motivation was that sweat sensing is limited not only by sensor chemistry, but also by the difficulty of generating, collecting, transporting, and refreshing very small volumes of sweat under realistic on-body conditions. Chapters 2–4 addressed this challenge from three connected perspectives: microfluidic transport design, electrochemical sensor integration, and on-body validation under low-flow and clinically relevant use cases.

5.1 Overall Summary

The first objective of this thesis was to establish a sweat-sampling architecture that can sustain fluid transport when sweat availability is low and time-dependent. Chapter 2 addressed this objective by developing a bioinspired microfluidic system based on directional interfaces and hierarchical transport pathways. The Janus inlet and outlet design promoted vertical sweat entry and removal, while the microchannel architecture supported lateral transport across the sensing region. Together, these design elements reduced stagnation and backflow and enabled more reliable sweat refreshment under conditions where conventional passive sampling approaches are less effective.

The second objective was to integrate this transport architecture with electrochemical sensing modules capable of monitoring metabolically relevant biomarkers. Chapter 3 addressed this objective by developing a multiplexed sensing array for uric acid, xanthine, alcohol, pH, and temperature. The enzymatic channels translated purine and alcohol-related metabolites into electrochemical signals, while the pH and temperature channels provided local environmental context needed for interpreting biochemical responses. Calibration, assay validation, reversibility, selectivity, shelf-stability, and auxiliary-sensor characterization showed that the sensing interface could support quantitative and multiplexed measurements before on-body use.

The third objective was to evaluate whether the combined microfluidic–electrochemical platform could operate under realistic wearable conditions. Chapter 4 addressed this objective through ultra-low-flow benchtop testing, PCB-integrated calibration, multi-day on-body monitoring, intake/activity studies, and gout-patient measurements before and after prescribed allopurinol intake. These studies demonstrated that the integrated system could maintain analyte refreshment and sensing under constrained sweat availability, follow dynamic purine-metabolism-related signals, and capture trends consistent with conventional blood-based measurements.

Overall, Chapters 2–4 show that continuous sweat monitoring requires a system-level approach. The microfluidic design determines whether fresh sweat reaches the sensing region, the electrochemical interface determines whether relevant biomarkers can be measured selectively, and the integrated wearable format determines whether these functions remain useful during real activity and clinical contexts. The thesis therefore advances wearable sweat sensing by linking induction, sampling, transport, sensing, and validation into a single platform.

5.2 System-Level Insights

A key insight from this work is that wearable sweat-sensor performance is governed by the coupling between fluid availability and sensor response. High sensor sensitivity alone is not sufficient if sweat is not continuously delivered to the sensing interface. Conversely, efficient transport has the greatest value when paired with sensing chemistries that can convert refreshed sample into interpretable biochemical information.

The results also show that low-flow operation should be treated as a design condition rather than a failure mode. Sweat secretion naturally decays after stimulation, and

wearable systems intended for practical monitoring must remain functional after the initial high-flow period. By improving collection, directional transport, and refreshment, the platform presented here extends the useful sensing window and reduces dependence on repeated sweat induction.

Finally, the thesis highlights the importance of multiplexed interpretation. Uric acid and xanthine provide pathway-level information about purine metabolism, alcohol offers behavioral and metabolic context, and pH and temperature help separate true biochemical changes from environmental effects on the sensors. This combination supports a more complete view of metabolic state than single-analyte monitoring alone.

5.3 Limitations

The present work establishes a platform-level foundation for continuous sweat monitoring, while leaving several opportunities for refinement. First, sweat secretion is inherently variable across individuals, body locations, stimulation conditions, and daily activities. The system was designed specifically to improve performance under low-flow conditions, and future studies can further define operating ranges across broader physiological contexts.

Second, the thesis uses iontophoresis to provide controlled and repeatable access to sweat. This approach is valuable for validating the sensing and transport platform because it creates a known sampling window and enables testing under delayed, low-flow conditions. Future versions may combine this capability with improved passive or minimally stimulated sampling modes to expand use during everyday sedentary conditions.

Third, the on-body studies demonstrate proof-of-concept performance in realistic and clinically relevant scenarios, including purine-metabolism monitoring and gout-patient measurements. Larger longitudinal studies would strengthen quantitative interpretation across diverse populations, diets, medication schedules, and disease states. These studies would build on the platform demonstrated here rather than changing the central contribution of the work.

5.4 Future Directions

The most important future direction is to use the microfluidic architecture as a versatile platform for broader metabolic monitoring. The transport system developed in this thesis is not limited to purine metabolites; in principle, the sensing layer

can be swapped or expanded while retaining the same sampling and refreshment strategy. This modularity creates a path toward monitoring additional biomarker panels relevant to metabolic syndrome, diabetes and its complications, hormonal regulation, inflammation, kidney function, and protein biomarkers.

For metabolic syndrome specifically, future sensor panels could combine purine metabolites with glucose, lactate, ketone-related markers, cortisol or other hormones, inflammatory proteins, and electrolyte/pH/temperature context. Such panels would move beyond a single disease marker and instead capture interacting pathways related to energy balance, oxidative stress, stress response, renal function, and lifestyle inputs. The same microfluidic platform could therefore serve as a general interface for studying how multiple physiological systems evolve over time in sweat.

Future work can also improve the analytical workflow around the wearable platform. Combining multiplexed sweat signals with calibration models, blood-reference datasets, and personalized baselines could help translate raw sensor outputs into more clinically interpretable trends. This will be especially important for biomarkers whose sweat–blood relationships depend on sweat rate, local gland metabolism, skin-surface accumulation, or transport delay.

From a device perspective, continued optimization of channel geometry, Janus interfaces, adhesive transport layers, and sensor packaging may further extend operation under ultra-low-flow or natural-sweat conditions. Integration with compact electronics and automated data processing would support longer monitoring durations and more user-friendly deployment.

Together, these directions position the platform as more than a single purine-metabolism sensor. The broader contribution is a modular sweat-sampling and sensing strategy that can be adapted to different biomarker chemistries, enabling future studies of metabolic syndrome and related chronic conditions through continuous, noninvasive, and system-level biochemical monitoring.

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