

Modular Biosensor Patch

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Principal Investigator:
Dr. Brian Henslee

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Cornerstone Research Group, Inc.
510 Earl Boulevard
Miamisburg, OH 4534217
(937) 320-1877 ext. 1210
hensleebe@crgrp.com

Contracting Officer's Representative:
Dr. Steve Kim

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Contractor Name: Cornerstone Research Group, Inc.
Address: 510 Earl Blvd, Miamisburg, OH 45342
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14. ABSTRACT

Report developed under SBIR contract for topic DHA18-003. Researchers are identifying new biomarkers to help monitor cognition and stress in the human body and enhance human performance. Traditional biomarkers like heart rate, temperature, oxygen saturation, blood glucose, electrolyte concentration, etc have been correlated with cognition and stress states. However, the correlation is indirect. Molecular biomarkers with stronger and more specific links are preferred. Molecular biomarkers can be more specific to cognition states and typically take the form of bioactive molecules including steroids, proteins, carbohydrates, lipids, and nucleic acids. The tools available to quantify molecular biomarkers during their discovery are sensitive and selective, but also have significant drawbacks. These tools are inconvenient in many ways and prohibit real time monitoring. As medical researchers identify new important molecular biomarkers, medical professionals need wearable sensor systems to take full advantage of their discovery. CRG plans to develop a modular wearable biosensor patch to provide non-invasive, continuous monitoring of sweat based biomarkers.

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1 INTRODUCTION

This report summarizes progress Cornerstone Research Group, Inc. (CRG) has achieved under contract W81XWH-18-C-0120 in a DHA Small Business Innovation Research (SBIR) Phase I project entitled, “Modular Biosensor Patch” awarded under 2018.1 SBIR solicitation, Topic DHA18-003, “Chronological Sweat Sensor Patch for Real-Time Human Molecular Biomarker Monitoring.”

Researchers are identifying new biomarkers to help monitor cognition and stress in the human body and enhance human performance. Traditional biomarkers like heart rate, temperature, oxygen partial pressure, blood glucose, electrolyte concentration, and others have been correlated with cognition and stress states. However, the correlation is indirect. Molecular biomarkers with stronger and more specific links are preferred. Molecular biomarkers can be more specific to cognition states and typically take the form of bioactive molecules including steroids, proteins, carbohydrates, lipids, and nucleic acids. The tools available to quantify molecular biomarkers during their discovery are sensitive and selective, but also have significant drawbacks. In particular, these tools are inconvenient in a variety of ways and prohibit real time monitoring. As medical researchers identify new molecular biomarkers of importance, medical professionals need wearable sensor systems to take full advantage of their discovery. CRG plans to develop a modular wearable biosensor patch to provide non-invasive, continuous monitoring of sweat based biomarkers.

2 OVERALL PROJECT SUMMARY

2.1 Executive Summary of Results

During this effort, CRG demonstrated feasibility of subsystems needed to realize a complete chronological sweat analysis and collection device.

Sweat control including aggregation, transition to a microfluidic system, and sequential filling of reservoirs was demonstrated at the bench level using synthetic eccrine sweat driven by a syringe pump. Design of the microfluidics included a study of multiple valve/control mechanisms before selection of capillary burst valves (CBV) as the primary flow control element. CBVs enable flow control without the need for cumbersome or bulky external drivers such as pneumatic pressure. Evaluation microfluidic devices were fabricated from polydimethylsiloxane (PDMS) for its flexibility and compatibility with aqueous systems like sweat and human skin contact. Sequential filling was demonstrated over six approximately 100 μ L reservoirs and evaluated multiple reservoir designs to target reliable and full filling. Sweat aggregation through a porous/breathable adhesive layer to a concentrated outlet point was demonstrated as a subsystem as well as with partial integration to a simple microfluidic channel to show transitions from sweat source, through an adhesive layer, and into the microfluidic sweat sensing and collection device.

An electronic circuit was designed and demonstrated for measurement of nanoamp current signals and integration as part of the sweat sensing and collection device. The circuit using a 24-bit analog to digital converter (ADC) was demonstrated in a breadboard configuration to resolve currents in microampere to nanoampere ranges, suitable for advanced sensors such as organic field effect transistors (OFET) as well as other types of sensors including electrochemical, potentiostatic, and resistive. The electronic measurement circuit demonstrated the ability to detect a sub-microamp signal from an OFET device when exposed to synthetic eccrine sweat.

Design efforts for an initial prototype design were conducted, producing models showing device integration from skin application to measurement electronics. This initial prototype design will form the basis for Phase II planning and refinement towards meeting Phase II objectives.

2.2 Individual Task Progress

Task 1 Chronological sweat collection fluidics design and prototype

Microfluidic design was developed leveraging capillary burst valves (CBV) and combined evaporative and perspiration pressure driving forces. These approaches have been documented in the literature ^[1,2] and offer the benefits of not requiring external support equipment to drive flow. The drawback, however, is the lack of or limited ability to directly control fluid flow. Initial designs were prepared for evaluation of CBV controlled flow with reservoirs of different sizes/volumes in the range of 0.5 μ L to 100 μ L (see Figure 1).

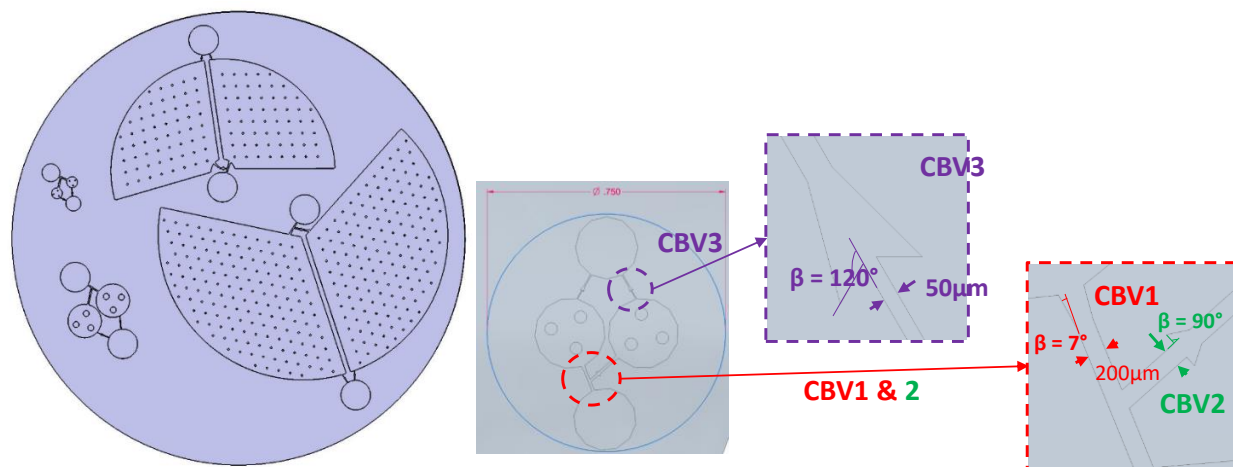


Figure 1: Initial microfluidic reservoir trial designs for four different volumes with detail of CBV features.

Analysis of microfluidic designs against the combined Phase I (time-stamped collection over 72 h, 100s of μL collected) and Phase II (50-100 μL per well collected, 2 inD x 0.125 inT device envelope) requirements for sweat collection revealed significant challenges. Using an estimate for sweat rate (thigh/arm of an active exerciser) of 0.6-1.1 $\mu\text{L}/\text{min}/\text{cm}^2$, a 2 inD area would have an effective sweat rate of 12.2-22.3 $\mu\text{L}/\text{min}$.^[4] This rate, if constant, would fill the target volume (6435 μL) of the entire device (2 inD x 0.125 inT) in 289-529 min, less than the 72 h goal. Depending on reservoir depth and design and PDMS layer thickness, the actual volume available to sweat storage would be significantly less than the full device envelope volume.

CRG considered concepts and approaches for active sweat flow control and diversion based on shunting flow to an evaporation chamber or overflow path. The main challenge here exists in controlling the diversion. Quake valves could be a solution for a limited number of control points.^[5] However, these valves would require pneumatic sources for actuation. Such sources could be used at the bench level for demonstration, but they would not fit within the device envelope outlined for Phase II. Alternatives in the literature included shape-memory material valves, phase change valves, and magnetic membrane valves.^[6-10] Conventional shape memory valves have the disadvantage of being single use which makes them unsuitable for cycling sweat collection on and off. Additionally, the heat necessary for initiating the memory effect would require substantial current which, while readily supported at bench scale, would be very challenging to support for long term operation from onboard stored energy. Phase change materials (e.g. paraffin wax) have essentially the same drawbacks as shape memory valves, requiring substantial heat to melt and being capable of only single activation. Magnetic membrane valves could be repeatedly actuated, but mechanical manipulation of permanent magnets would be very challenging within the target device envelope and would entail significant concerns about reliability under dynamic conditions. Use of an electromagnet would require very significant power to achieve the necessary magnetic flux to actuate this type of valve, effectively arriving at a similar drawback as the conventional shape memory valves.

CRG considered several algorithms for sweat collection, depicted in Figure 2. Scheme (a) had the advantages of direct time stamping and discrete samples, but its disadvantages included greatest complexity, need for a controllable and reversible set of valves, likely high power demand and/or supporting actuation infrastructure for such valves, and uncertain capacity capability. Scheme (b) had advantages of discrete samples of sweat with the addition of simpler microfluidic design and control, but its disadvantages included variable time stamping and sweat collection rate dependent upon subject perspiration rate. Scheme (c) had as its advantage great simplicity, but its disadvantages included those of (b) and additionally a necessity for plug flow to avoid sweat sample mixing. In light of the assessments of microfluidic valve concepts and sweat collection algorithm, CRG elected to proceed down a path like scheme (b), which was also analogous to an approach demonstrated by the Rogers group at Northwestern University.^[1]

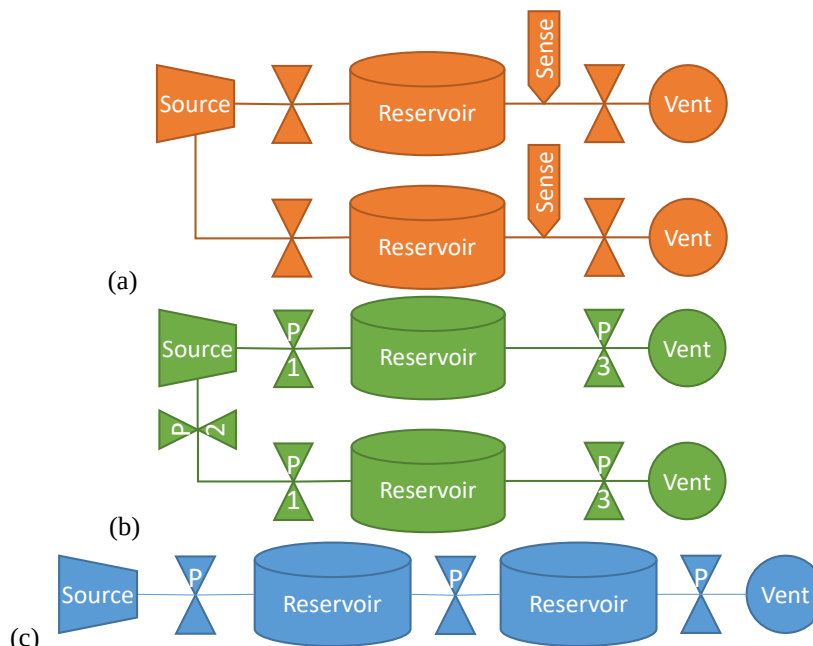


Figure 2: Sweat collection algorithms: (a) discrete collection with active/direct on/off control for each reservoir, (b) continuous collection with passive control of flow to each reservoir, and (c) continuous first in – first out flow with little to no control.

CRG prepared different microfluidic designs for demonstration of sequential sweat collection and for evaluation of various integration criteria. The initial design shown in Figure 1 was used for vetting of the CBV approach and initial assessment of reservoir filling. That approach was expanded to a full 2 in diameter collection reservoir design (see Figure 3) targeting storage of several hundred microliters of sweat in six reservoirs. This design pushed toward Phase II objectives with respect to device envelope and reservoir size, while also addressing the Phase I requirement of demonstrating hundreds of microliters of sweat collection. Additional designs included a simple microfluidic channel for assessing feasibility of sweat transition from simulated source to microfluidic system and for early investigation of a concept for sweat rate and time indexing. These devices are shown in Figure 4.

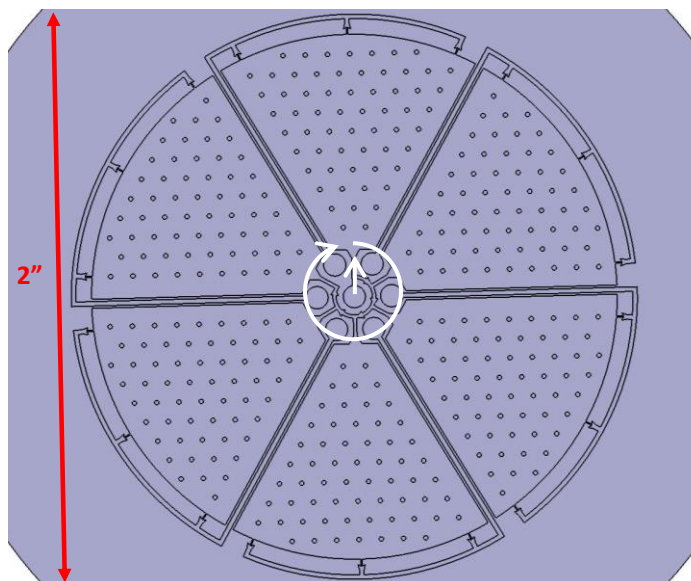


Figure 3: Design targeting 100s of microliters of sweat collection. Each reservoir should contain $\sim 98 \mu\text{L}$, with six reservoirs totaling $\sim 590 \mu\text{L}$ total.

During this reporting period, CRG began testing microfluidic devices leveraging capillary burst valves (CBV) demonstrating various subcomponents for integration into a complete sweat sensor during Phase II. These designs included (see Figure 7):

1. one design with several reservoirs of varying sizes used for test development
2. a second design with six 100 μL reservoirs for demonstration of serial sweat storage using passive controls (i.e. CBV)
3. a third design with several chained reservoirs for demonstration of sweat rate monitoring through an external resistor network
4. a fourth design with a simple microfluidic channel including a window for integration of a sensor for demonstration of sensor measurement feasibility.

Demonstration of reservoir filling was conducted with designs #1 and #2 using synthetic eccrine sweat (Pickering Laboratories Artificial Perspiration 1700-0020) dyed with green food coloring for contrast. The colored synthetic sweat was connected to the microfluidic devices using fine gauge tubing and needles and/or ports embedded into the PDMS. Sweat flow was controlled using a syringe pump (Chemyx Fusion One). During demonstration, microfluidic behavior was monitored with photographs and video macroscopically and microscopically.

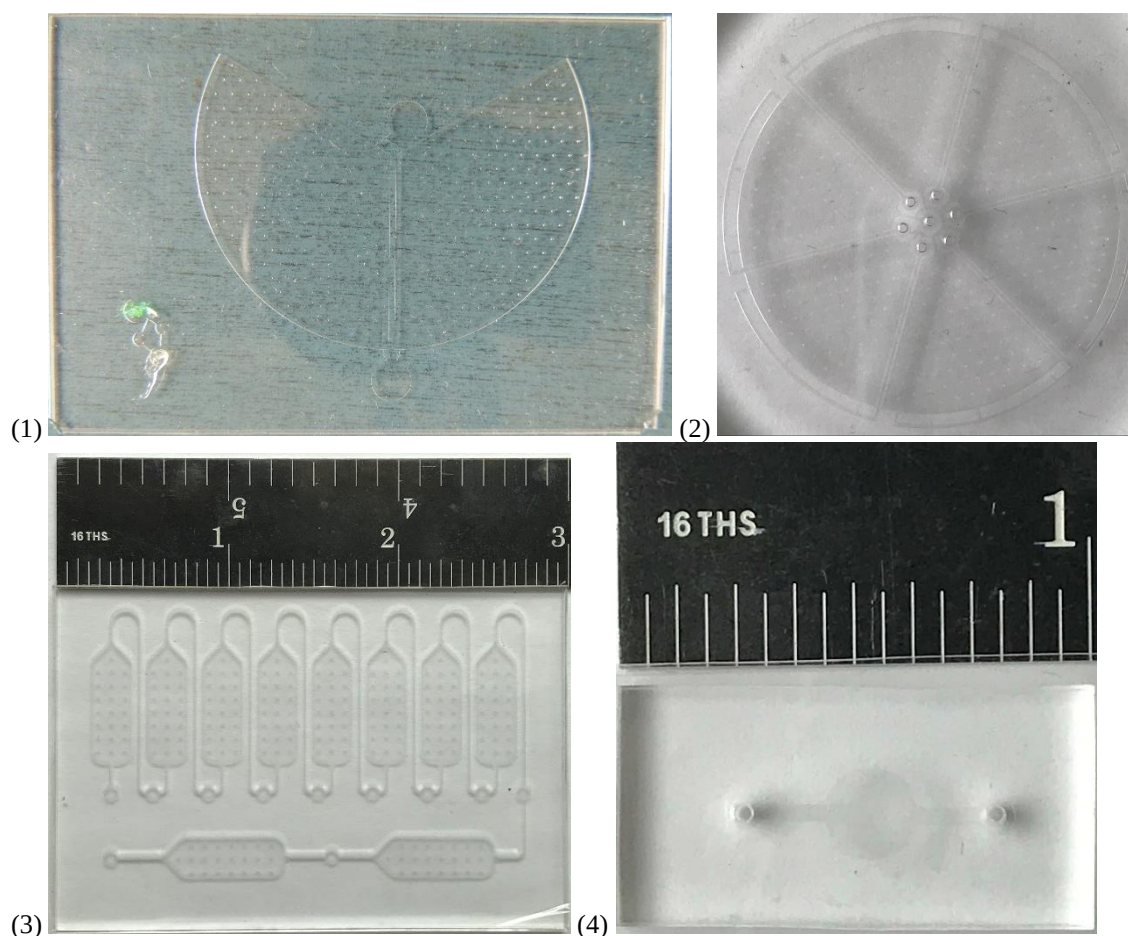


Figure 4: Microfluidic demonstration designs – (1) test development design with different reservoir sizes, (2) 6x100 μL reservoir and CBV demonstration design, (3) sweat rate measurement demonstration design, and (4) sensor integration demonstration design

CRG evaluated sweat reservoir filling using dyed synthetic eccrine sweat (Pickering Laboratories Artificial Perspiration 1700-0020). The colored (green) synthetic sweat was connected to the microfluidic device using fine gauge tubing/needle and ports embedded into the PDMS. Sweat flow was controlled using a syringe pump (Chemyx

Fusion One). During demonstration, microfluidic behavior was monitored with photographs and video. The port(s) on the device were included to aid in the evaluation of the system, but they would not be part of the integrated Modular Biosensor Patch.

Time lapse frame captures of reservoir filling are shown in Figure 5. Sequential filling of the reservoirs was successful, with each successive reservoir only beginning to fill after no more sweat could be added to the preceding reservoir. Of the six reservoirs, only one filled to completion; the other five each entrained an air pocket of various size. As can be seen from the more detailed frame captures (i.e. a through f) of the first reservoir filling, the sweat tended to flow initially along the continuous wall of the reservoir, with limited ingress through the array of reservoir support columns. This indicated some affinity of the sweat to flat surfaces within the reservoir over curved surfaces such as the columns. Effectively the support columns acted as an array of gates or CBVs impeding the uniform inflow of sweat in the reservoir. The severity of the air entrapment appeared to be variable and the product of stochastic progress of the sweat through the support column array.

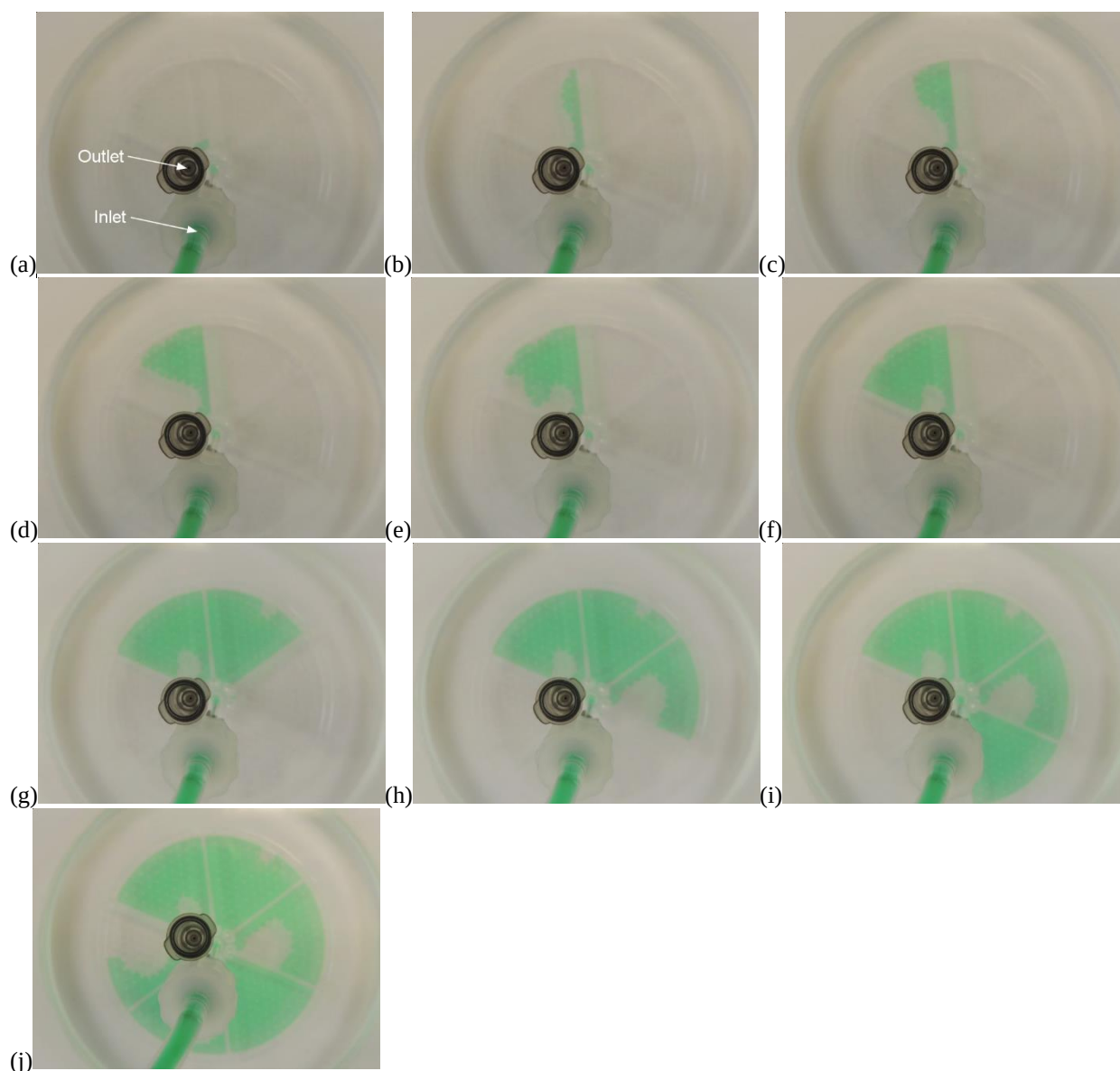


Figure 5: Time lapse of reservoir filling using design #2: (a) 0 min, (b) 1.5 min, (c) 3 min, (d) 4.5 min, (e) 6 min, (f) 7.5 min, (g) 15.75 min, (h) 18.6 min, (i) 23 min, and (j) 28.4 min

The syringe pump was initially configured for a slow flow rate of approximately 11.5 $\mu\text{L}/\text{min}$. This nominal flow rate was used for the first three reservoirs of the experiment. After this midpoint, the flow rate was doubled to nominally 23 $\mu\text{L}/\text{min}$ for the remaining reservoirs. This increase was implemented to accelerate the demonstration. A summary of reservoir filling statistics is shown in Table 1. Allowing for errors in determining the end of reservoir filling from video and the effect of trapped air pockets, the effective flow rates were in reasonable agreement with nominal rates, except for the third reservoir. The table lists reservoirs five and six combined because the start and end of filling in reservoir five could not be observed from the video and because reservoir six was incompletely filled by the end of video monitoring.

Table 1: Summary of reservoir filling statistics
** Reservoir 6 did not fill completely by the end of the monitoring video*

Reservoir	Nominal Flow Rate, $\mu\text{L}/\text{min}$	Total Elapsed Time, min	Incremental Elapsed Time, min	Effective Flow Rate, $\mu\text{L}/\text{min}$
1	11.5	7.5	7.5	13.3
2	11.5	15.75	8.25	12.1
3	11.5	18.6	2.85	35.1
4	23	23	4.4	22.7
5+6*	23	28.4	5.4	27.8

Micrographic study of reservoir filling (see Figure 6) indicated a surface tension effect between sweat, the reservoir support columns, and the unfilled reservoir volume. To overcome this effect, CRG revised the sweat collection reservoir design to evaluate alternative reservoir support concepts to mitigate and/or eliminate the propensity to entrap air within the reservoir, limiting sweat collection, and resulting in variable filling time for each reservoir.



Figure 6: Micrograph illustrating the surface tension effect of sweat passing between reservoir support columns

Demonstration of reservoir filling was conducted with revised reservoir design (see Figure 7) as before. Time lapse frame captures of reservoir filling are shown in Figure 8. Sequential filling of the reservoirs was demonstrated through five reservoirs, with each successive reservoir only beginning to fill after no more sweat could be added to

the preceding reservoir. The first four reservoirs exhibited a high degree of filling, demonstrating improvement over the previous demonstration. The serpentine pattern showed essentially perfect filling, representing a promising path for reliable reservoir volumetric filling. The summary of reservoir filling in Table 2 shows good agreement between effective rate of sweat filling in the reservoirs and nominal flow rate from the syringe pump. Variations could be attributed to deviations of actual reservoir volume from nominal and uncertainty with respect to reservoir filling start and end times.

The demonstration failed in the fifth reservoir with sweat bursting the exit/extraction valves of the first reservoir. This event has been explained as high back pressure resulting from the dense rib pattern included in reservoir five. The survey – for alternative reservoir support designs – nature of these designs makes firm conclusions regarding the relative influence of downstream reservoirs challenging. However, feasibility of full filling has been demonstrated with the serpentine design. Further optimizations around that reservoir support concept and capillary burst valves could be accomplished during Phase II, along with additional investigations to optimize reservoir design/performance over a range of sweat rates.

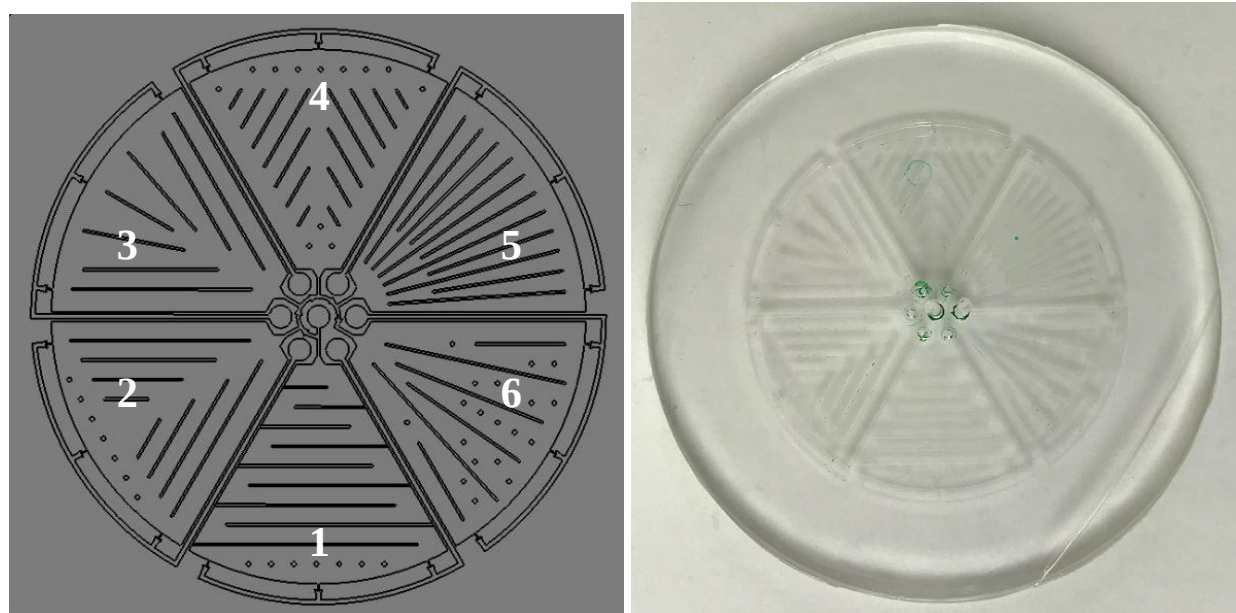


Figure 7: Microfluidic revised demonstration design: drawing (left) and received part (right)

Table 2: Summary of reservoir filling statistics
* Reservoirs 5 and 6 did not fill completely due to reservoir 1 exit CBV bursting

Reservoir (Volume)	Nominal Flow Rate, $\mu\text{L}/\text{min}$	Total Elapsed Time, min	Incremental Elapsed Time, min	Effective Flow Rate, $\mu\text{L}/\text{min}$
1 (97 μL)	25	3.167	3.167	30.6
2 (99 μL)	25	6.333	3.167	31.3
3 (100 μL)	25	10	3.667	27.3
4 (100 μL)	25	13.667	3.667	27.3
5* (94 μL)	25	N/A	N/A	N/A
6* (99 μL)	N/A	N/A	N/A	N/A

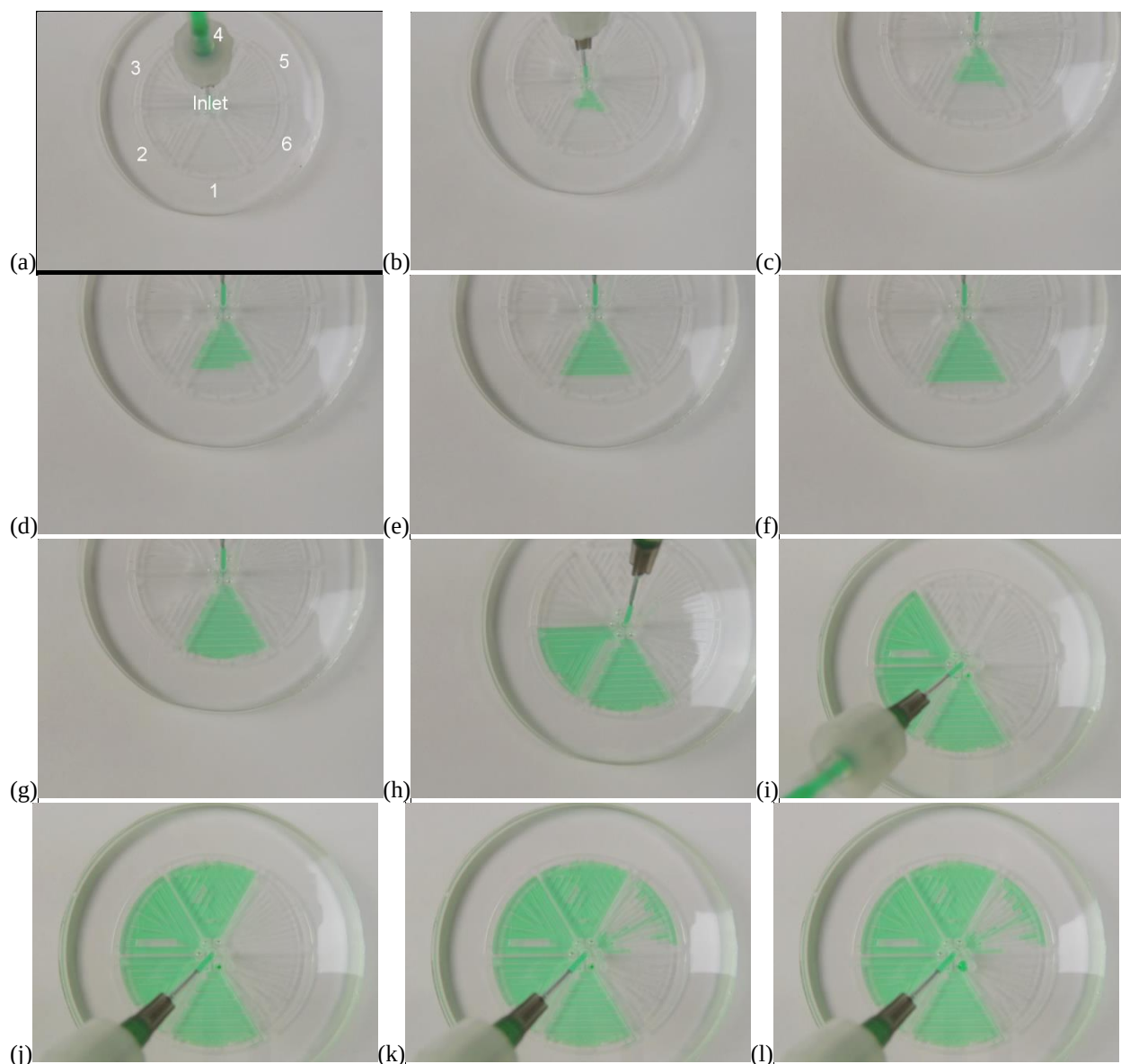


Figure 8: Time lapse of reservoir filling using revised reservoir design: (a) 0 min, (b) 0.5 min, (c) 1 min, (d) 1.5 min, (e) 2 min, (f) 2.5 min, (g) 3.167 min, (h) 6.333 min, (i) 10 min, (j) 13.667 min, (k) 15 min, and (l) 15.333 min.

Demonstrations were conducted for aggregation of sweat from an area to an outlet. The first trial utilized filter paper (Whatman) as a porous aggregation medium. The paper was masked with clear tape into which a hole was punched to represent the outlet. The filter paper was supported against a glass plate using the clear tape. Dyed synthetic eccrine sweat was introduced at one location of the filter paper and sweat exited through the outlet hole upon saturation of the paper (see Figure 9).

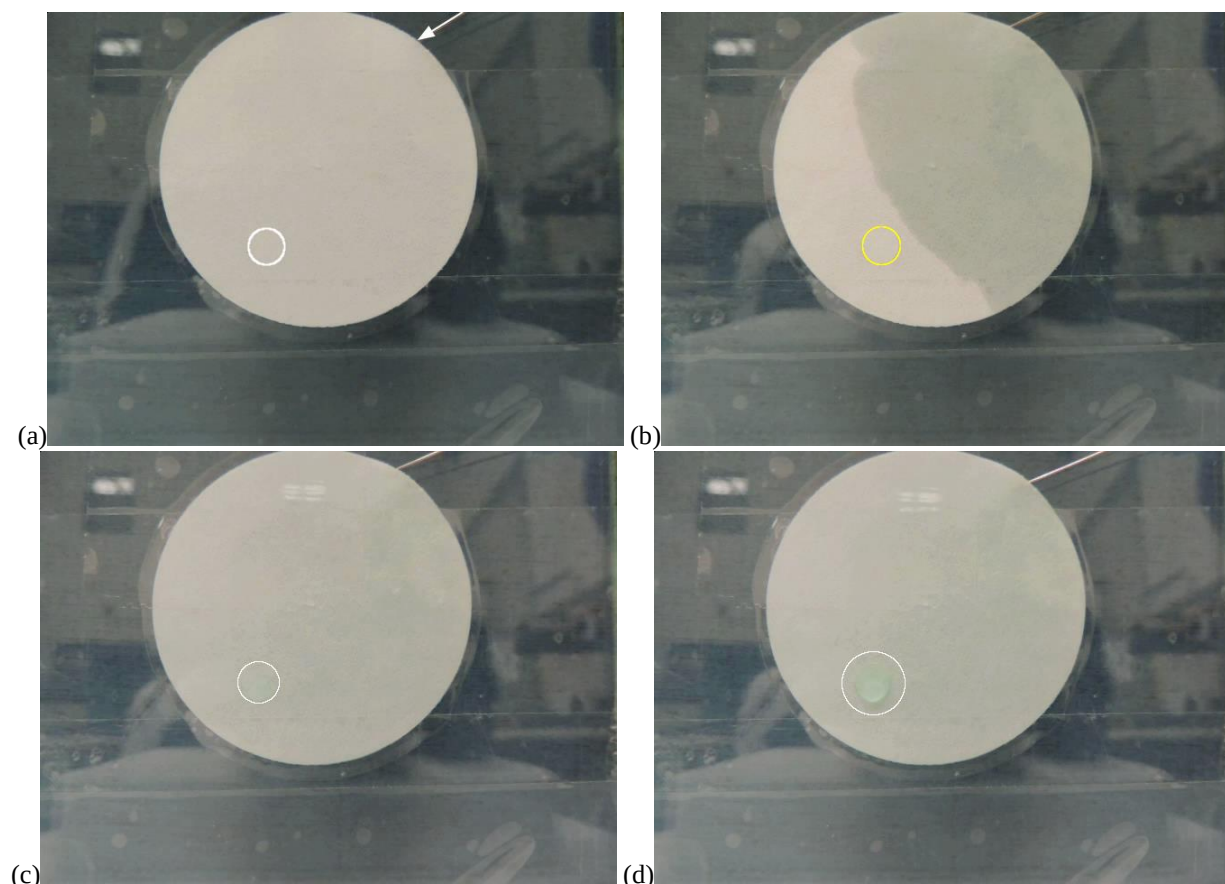


Figure 9: Time lapse of sweat aggregation demonstration using filter paper: (a) completely dry at 0 s, (b) partially filled at 23 s, (c) fully filled at 43 s, and (d) oversaturated with fluid exiting at 53 s

A recognized drawback of using filter paper was the significant pore volume which had to be saturated prior to there being a driving force for fluid flow into a microfluidic device. Having demonstrated gross feasibility of sweat aggregation using the simple yet effective filter paper medium, CRG conducted an additional trial with a reduced area/volume of porous filter paper needed to direct sweat to an outlet. The reduction was demonstrated by cutting/patterning of the filter paper into an approximation of a hub-and-spoke design. The second demonstration was similar to results reported in the first demonstration. Differences included: reduction of the filter area to mitigate dead zones, reorientation of the test horizontally rather than vertically, inclusion of a breathable adhesive layer between filter paper and sweat source, and use of a porous stainless steel disc for diffusion of sweat flow (approximation of skin pores rather than a directed point source of sweat.). Time lapse results are shown in Figure 10. Compared to the previous demonstration, this setup integrated an adhesive layer with the proposed distribution layer and introduced the sweat more indirectly to show that the proposed concept is feasible in the context of dermal contact. An important observation was that once the synthetic sweat began wetting the filter paper aggregation layer, further saturation of the breathable adhesive layer effectively stopped as the sweat was directed to and exited the outlet orifice of this demonstration.

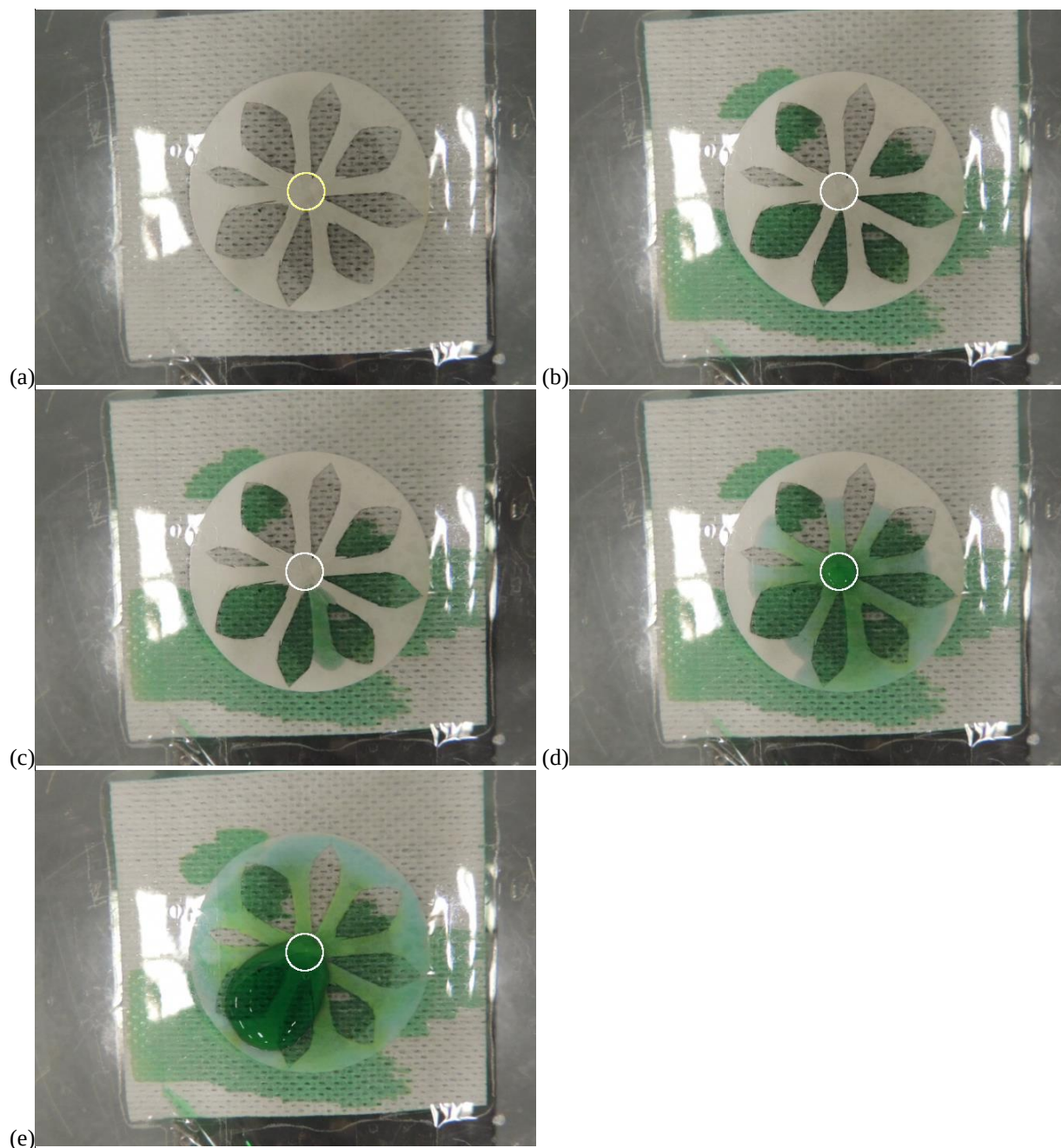


Figure 10: Time lapse of sweat flow demonstration through porous/breathable adhesive layer and reduced volume filter paper: (a) start of test at 0 s, (b) initial flow before filter wetting at 188 s, (c) fluid spreading at 193 s, (d) sweat bead forming at exit at 226 s, and (e) end of test at 476 s

Another demonstrate focused on showing integration between sweat aggregation and microfluidic device. A similar assembly of porous metal diffusion disc and breathable adhesive were used along with a simple microfluidic channel device to show sweat flow transitioning between aggregation and microfluidics. Time lapse results are shown in Figure 11. As the demonstration progressed, sweat was observed to enter the microfluidic channel, filling narrow trace and round reservoir area, and then proceeding to the microfluidic outlet. While the breathable adhesive layer exhibited spreading of the sweat throughout the demonstration, this was the result competition between the resistance of the microfluidic channel and large open area of the breathable adhesive layer. In application, the spread in the breathable adhesive layer would be mitigated through constraint of the layer area and/or more tailored porosity of the layer.

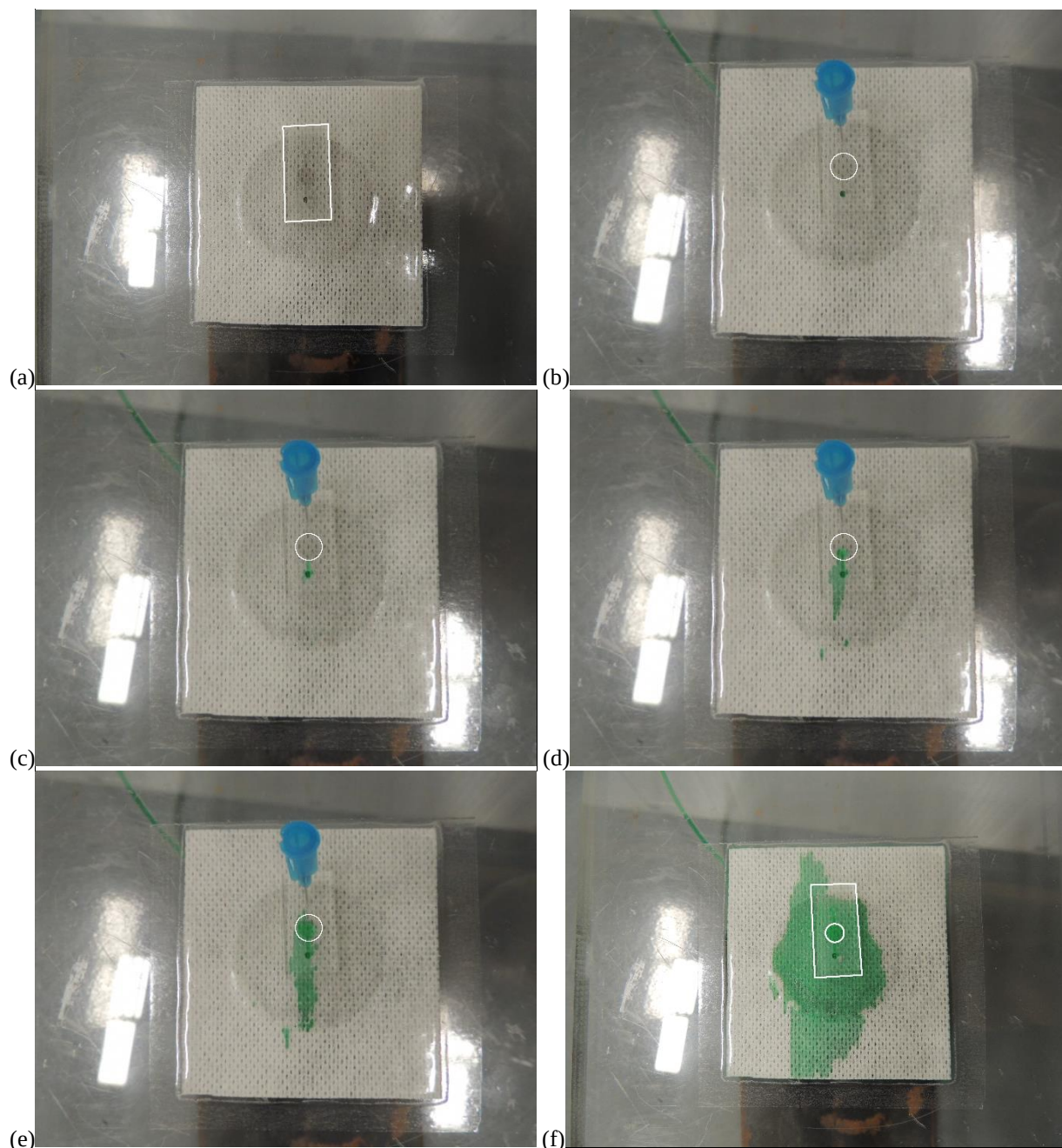


Figure 11: Time lapse of sweat flow demonstration through porous/breathable adhesive layer to PDMS μ -fluidic channel: (a) pre-test, (b) start of test at 0 s, (c) initial filling at 3 s, (d) partially filled at 9 s, (e) fully filled at 18 s, and (f) post-test

CRG evaluated a concept for sweat rate estimation using probes embedded at different positions in a microfluidic channel. In this concept, the assumption was to use the sweat (in this case synthetic eccrine sweat) as an electronic switch to alter the effective resistance of a resistor network. As sweat would progress from probe to probe, another resistor would be added to the network, changing the effective resistance in a predictable way. This concept was attractive as an initial approach due to its indirect measurement of sweat rate based on detected sweat progress and known fixed volumes filled. However, in testing of this concept using a demonstration microfluidic design, the impedance of the synthetic eccrine sweat was sufficiently high that its behavior was not like an electronic

switch. The evaluated approach to sweat rate estimation may yet be feasible with reconfiguration during a Phase II effort. Alternatively, capacitive behavior may be another approach to pursue for sweat rate estimation.

From the effort of this task, CRG has demonstrated the feasibility of passive flow control of sweat within a microfluidic storage platform capable of containing multiple 100 μL of sweat. Full filling of these reservoirs was shown to be attainable with appropriate internal design. Aggregation and direction of sweat from a broad area to desired outlet and transition to a microfluidic device was demonstrated through integration of porous adhesive layer, porous filter paper for aggregation, and simple PDMS microfluidic channel. The optimization and integration of these core elements during a Phase II effort presents the opportunity for an effective, small form factor sweat collection device.

Task 2 Electronics design and prototyping

An electronic circuit capable of measuring small signal levels was developed for integration with the microfluidic sweat collection device. The circuit was designed with the intention to pair with various sensor types to realize modularity of the overall device to different sweat analytes. The circuit was further demonstrated as a bread board assembly at the bench level.

CRG began this effort by identifying components and subcircuits with which to accomplish measurements at low signal (e.g. current) levels from biosensors (e.g. OFET). In addition to low signal capability, power consumption was another factor which figured into selections. It was desirable to utilize low power components as much as possible in order to achieve long device run time while minimizing mass and volume required for energy storage (i.e. batteries).

The desired solution for achieving sensor signal acquisition was an integrated device (i.e. chip) incorporating both analog and digital (ADC and DAC) functionalities with a microcontroller. An integrated device could minimize power consumption. ADC resolution of 16- or 24-bit or greater was determined to be necessary to enable direct low signal capture, but lower resolution may be feasible with amplification. The convenience of available preassembled demonstration/evaluation circuit boards for easier feasibility assessment and development was also desirable but not necessary. CRG examined four microcontroller options, each of which was lacking in one or more areas described above. One of these options when coupled with additional components for analog to digital conversion could match all of the characteristics with the potential to eliminate the additional components if lower ADC resolution were feasible. This multicomponent approach was selected as the most viable path forward. After procuring components with demonstration boards, physical interconnections were established (see Figure 12) and microcontroller software controlling the flow of signal/data between components was developed using a simulated input in the form of a photodiode signal.

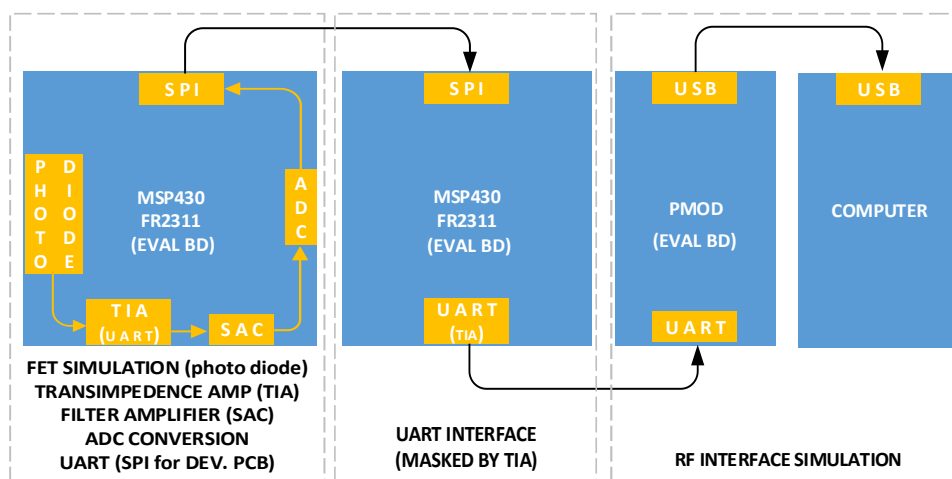


Figure 12: Measurement circuit block diagram with component interconnection.

The circuit shown in Figure 13 was comprised of a primary microcontroller located on the right-hand red board. This microcontroller (Texas Instruments MSP430FR2311) combines functions for measurement control and communications by featuring one trans-impedance amplifier for current to voltage conversion, one general purpose operational amplifier for filtering, and a 10-bit analog to digital converter (ADC) for conditioning and acquiring the sensor response. The green board at the top of Figure 13 is an alternative analog-to-digital converter with 24-bit resolution (Texas Instruments ADS1246). The combination of the two converters offers flexibility for different

levels of signal. The left-hand red board is identical to the already described right-hand board. The function of the left-hand board in this demonstration was only to provide an interface to a PC for data logging. In the final configuration, this board was replaced by an RF transceiver (Texas Instruments CC1101) evaluation board to enable wireless communications using the same SPI communications bus and configuration with the 24-bit ADC.

With measurement circuit functionality established, a demonstration was conducted with a signal coming from an on board photodiode from the development board. This signal was processed through the same circuit and software designed for monitoring of a sweat analyte sensor. The side-by-side outputs of 10-bit and 24-bit resolution ADCs are shown in Figure 14. The vertical axes in those charts represent full scale of the respective ADC. As was expected, the two ADCs returned comparable values relative to their scales of resolution. In a real device with an active sensor, these scales would be converted and calibrated to reflect the a physical quantity being sensed.

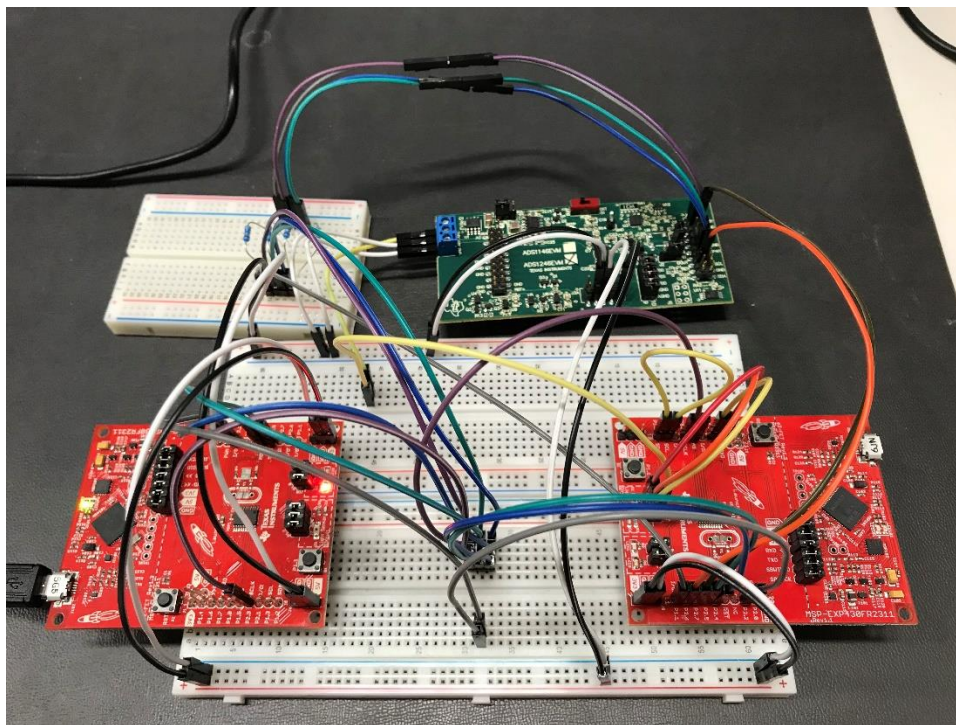


Figure 13: Measurement circuit using demonstration boards

A review of literature was conducted to assess how different types of sensors may fit within the measurement platform. Current response from sensors for glucose and lactate have demonstrated levels in the range of hundreds to thousands of nanoamperes.^[11] Aptamer-based sensors for neuropeptide-Y have been demonstrated with current signals on the order of hundreds of nanoamperes, similar to that of some glucose and lactate.^[12] The present measurement circuit being developed should have resolution with the 24-bit ADC of 60-200 nA, feasible to make measurements for very low level signals. Other sensors for electrolytes such as Na⁺ and/or K⁺ with voltage signals on the order of hundreds of millivolts^[11] fall well within the resolution of even the current 10-bit ADC whose resolution extends down to ~1 mV. Similarly, resistive signal sensors for temperature could be integrated with high resolution enabled by the combination of the transimpedance amplifier and ADCs.

The intent for development of the measurement circuit was to enable the concept of a modular sweat sensor patch. As indicated by the review of literature for sensors relevant to sweat analyses, the current circuit would enable such modularity, being essentially sensor (and signal) agnostic with easy adaptability to different sensors. This modularity will enable the possibility to integrate different sensor for analytes of interest into the sweat collection and analysis framework of the modular biosensor patch under development.

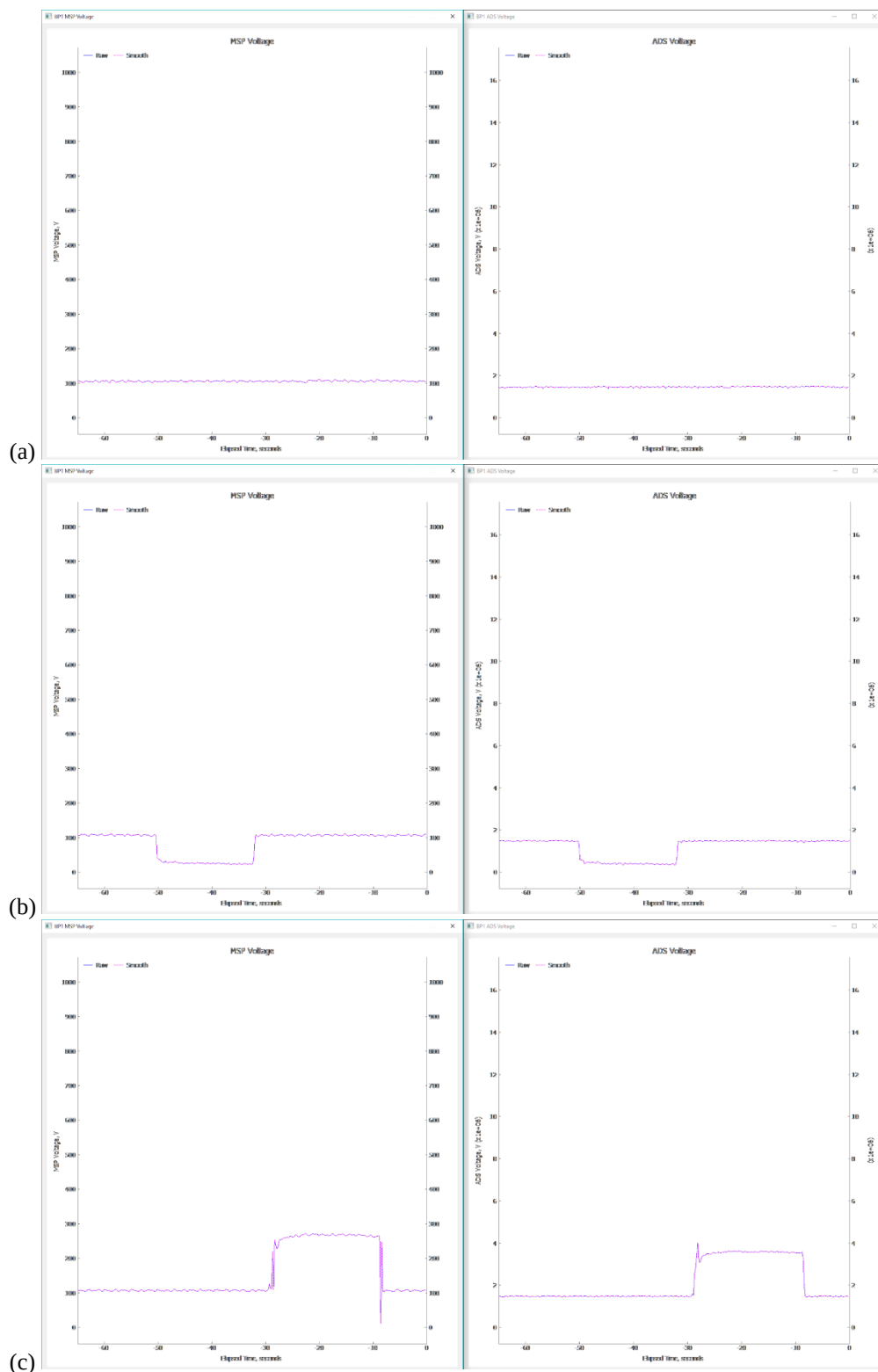


Figure 14: Charts of measurement data using CRG developed measurement circuit (10-bit ADC on the left and 24-bit ADC on the right) with synthetic signal: (a) baseline, (b) negative change and return to baseline, and (c) positive change and return to baseline

Building on the initial successful demonstration of the measurement circuit with a synthetic signal, CRG demonstrated signal response and measurement using a generic OFET device and synthetic eccrine sweat. The circuit was the same as shown in Figure 13 and the OFET test bed socket is shown in Figure 15. The OFET testbed was leveraged from a previous SBIR Phase II program with AFRL (Contract: FA8650-16-C-5410). This demonstration monitored the OFET device current with the measurement circuit initially under dry conditions to establish a baseline, followed by the introduction and removal of synthetic eccrine sweat as well as deionized water. Measurement results are shown in Figure 16. The data exhibit spikes at the introduction and removal of sample fluids. These peaks are related to disturbance of the device and capacitive responses. After establishing the baseline current, a drop of synthetic eccrine sweat was introduced onto the active device area, resulting in a signal response as indicated in Figure 16. Removal of the synthetic sweat produced a return to the baseline current. A second introduction of synthetic sweat produced similar results as the first drop. After again removing the sweat and returning to the baseline, a drop of deionized water was introduced, producing negligible response.

The response of the measurement circuit exhibited different degrees of clarity depending on which resolution of analog-to-digital converter (ADC) was examined. The signal responses were clear and readily apparent in the 24-bit ADC results. However, there was significant noise in the 10-bit ADC results. A running average analysis elucidated the signal above the baseline, but this approach would not be conducive in a fielded device. Using the higher resolution 24-bit ADC presents the most direct route to capturing low level signals, like the sub-microamp signals captured in this demonstration.

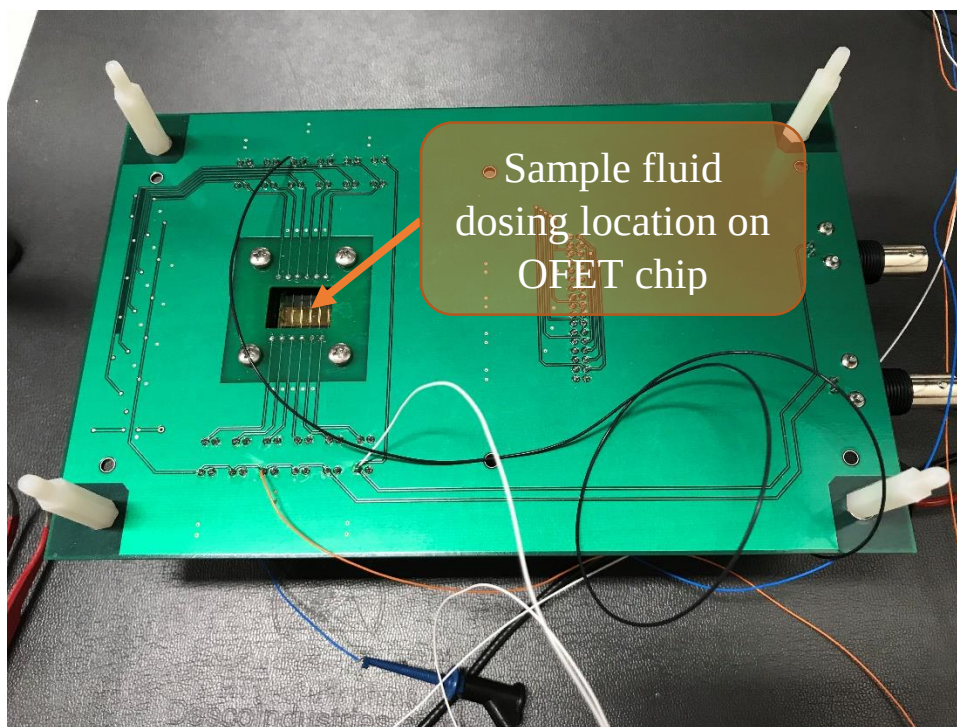


Figure 15: Measurement circuit using demonstration boards (upper) and OFET test bed interface (lower)

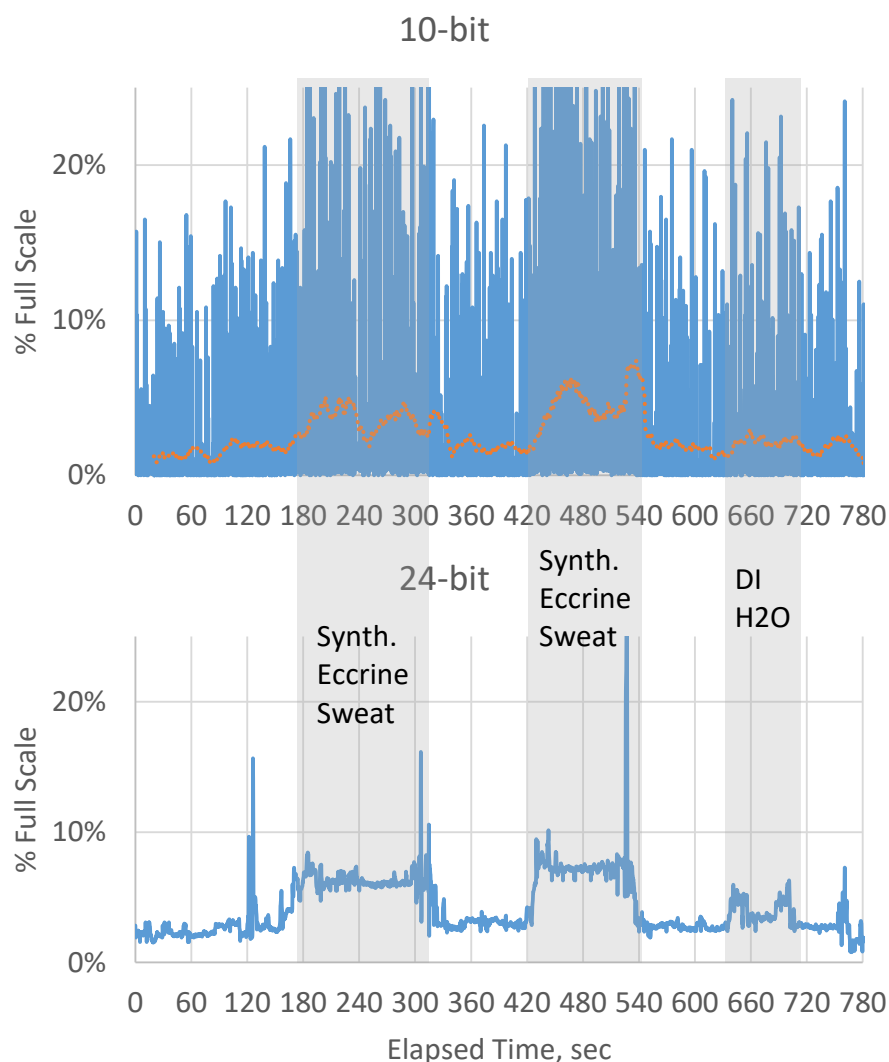


Figure 16: Charts of signal response using CRG developed measurement circuit (10-bit ADC upper and 24-bit ADC lower) with synthetic eccrine sweat and DI water

One of the targets for component selection in this task was low power consumption. An assessment of runtime with different measurement duty cycles was performed to estimate required stored energy to achieve the 72 h operation target. Based on conservative current levels from the component data sheets, a measurement cycle build-up was constructed. This build-up estimated a wake-measure-transmit cycle to take just over 1 s to complete and to consume 1.4 μAh of energy. Table 3 summarizes the estimated total energy consumption of the measurement circuit for different measurement intervals ranging from 2 s to 60 s. The required energy decreases with longer intervals between measurements as more time is spent by the electronics in standby/sleep state. The low energy cost of operation achievable with this circuit allows for the possibility of utilizing thin, light-weight batteries (e.g. BrightVolt lithium polymer, see Figure 17) to power the modular biosensor patch. By stacking multiple of these cells in parallel, sufficient capacity is achievable to support the various measurement periods shown in Table 3.

Table 3: Summary of required energy over 72 h operation for different measurement intervals

Measurement Cycle Period, s	2	5	10	15	30	60
Estimated Total Capacity for 72 h runtime, mAh	190	83	47	35.3	23.5	17.5
Estimated Number of Parallel Cells (25 mAh each)	8-9	4	2-3	2	1-2	1

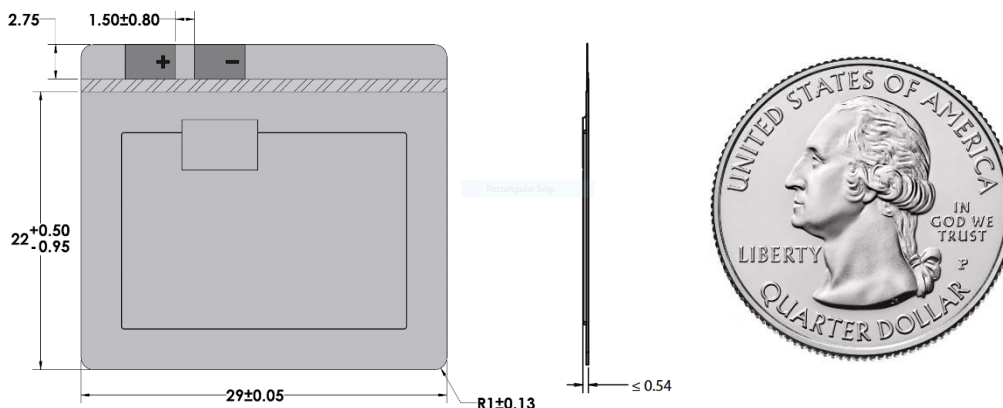


Figure 17: Comparison of 25 mAh BrightVolt cell (dimensions in mm) to a US quarter.

From the effort of this task, CRG has demonstrated the feasibility of measuring low level signals to enable broad modularity of the sweat sensor toward various analytes of interest. While this feasibility demonstration has shown very real promise with a nominal OFET sensor and synthetic sweat, more rigorous evaluation with sensors of different types (e.g. OFET, electrochemical, resistive, etc) is needed as part of a Phase II effort. Additionally, migration of the design from bread board and demonstration boards to an application dedicated layout and fabricated device will be needed during a Phase II effort.

Task 3 Design integrated sensor device

Based on results of feasibility demonstrations in Task 1 and Task 2, CRG conducted preliminary design work for an initial prototype integrated sweat sensor patch. Sweat aggregation, microfluidics, sweat collection, and electronics concepts and components demonstrated during this Phase I effort have been incorporated into this initial design. Features lending toward modularity have also been incorporated. A rendering of this prototype integrated device is shown in Figure 18. This design is shown in exploded view in Figure 19 and in cross-section view in Figure 20. Additional images of individual layers are shown in Figure 21, Figure 22, and Figure 23.

This initial design as shown has thickness estimated at 3530 μm (0.139 in). The goal for the design is to meet the overall thickness target with maximized sweat collection volume. While this is slightly thicker than the Phase II target thickness of 0.125 in, the present design includes a 790 μm thick sensor layer that could be optimized down to 400 μm along with other layer thickness reductions to achieve the Phase II envelope. The prototype design shown is based on design rules and assumptions based on Phase I demonstrations. During a Phase II effort, these design rules and assumption could be improved and revised. Without any of that work, the present design could comply with the thickness target by removing one of the two sweat reservoir layers. Alternatively, optimizations and/or revision of assumptions for the sensor layer could reduce the thickness of the device to the target thickness. Enhancement of the sweat reservoir layer design to enable stacking of multiple instances of the same layer is currently projected to reduce the reservoir volume. However, inspection of the design (see Figure 23) reveals that there is available space which could be used to restore reservoir volume.

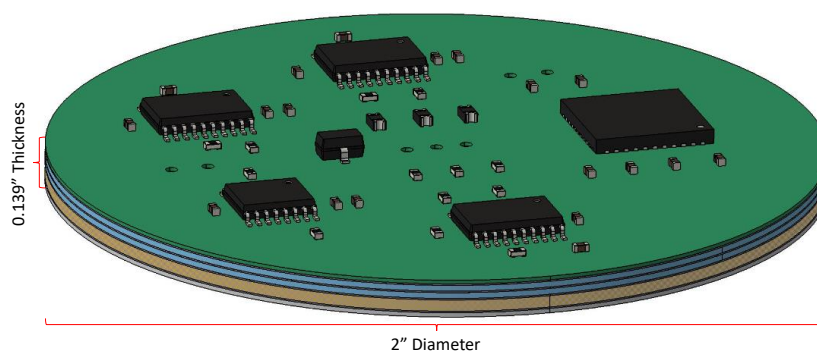


Figure 18: Rendering of initial prototype integrated sweat sensor patch design: gray (bottom) layer is medical grade adhesive, yellow layer is for sensor integration, blue layers are for sweat collection, and green layer is electronics for the sensor.

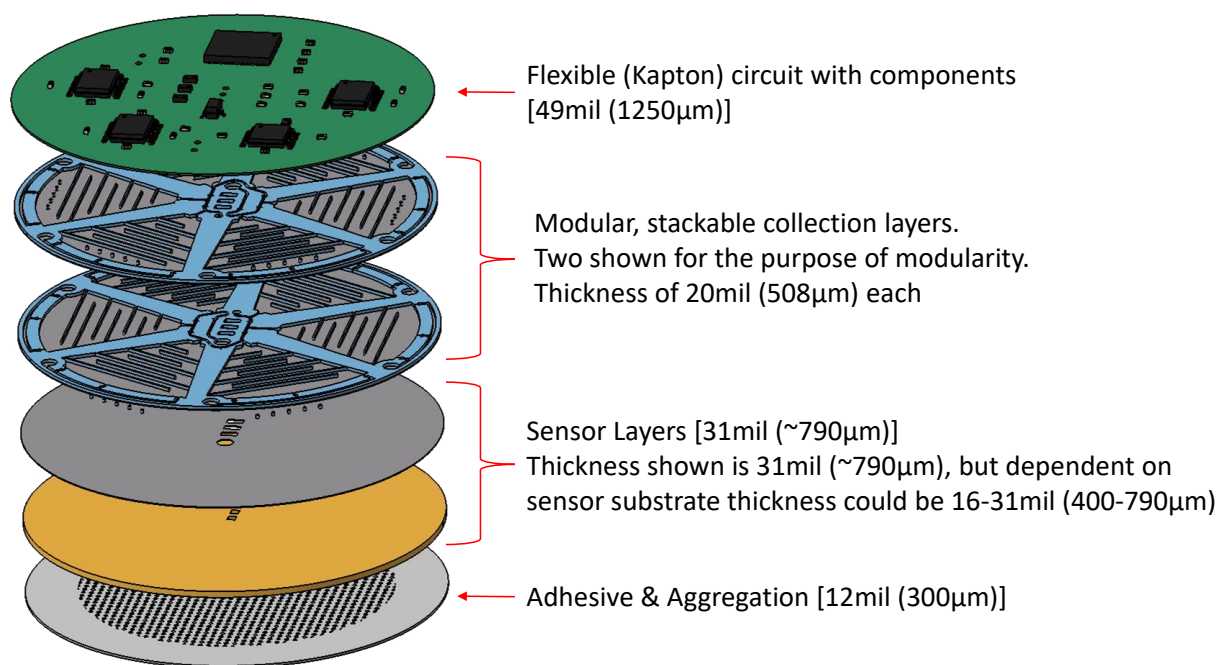


Figure 19: Exploded view of initial prototype design

As shown in Figure 20, sweat driven by gland pressure would enter the device and progress across layers before proceeding up to the next layer. After aggregation, sweat would pass through a sensor element embedded within the sensor layer. This layer as shown (see Figure 22) underutilized the space available. This was intentional to enable sensor modularity. Minor modification of this layer design would enable integration of sensors for different analytes with variable areal dimensions without necessarily affecting sweat collection or electronic layers higher in the stack. After analyzing the sweat, flow continues into a reservoir/collection layer where the sweat fills reservoirs one-by-one in sequence before moving up to additional reservoir collection layers.

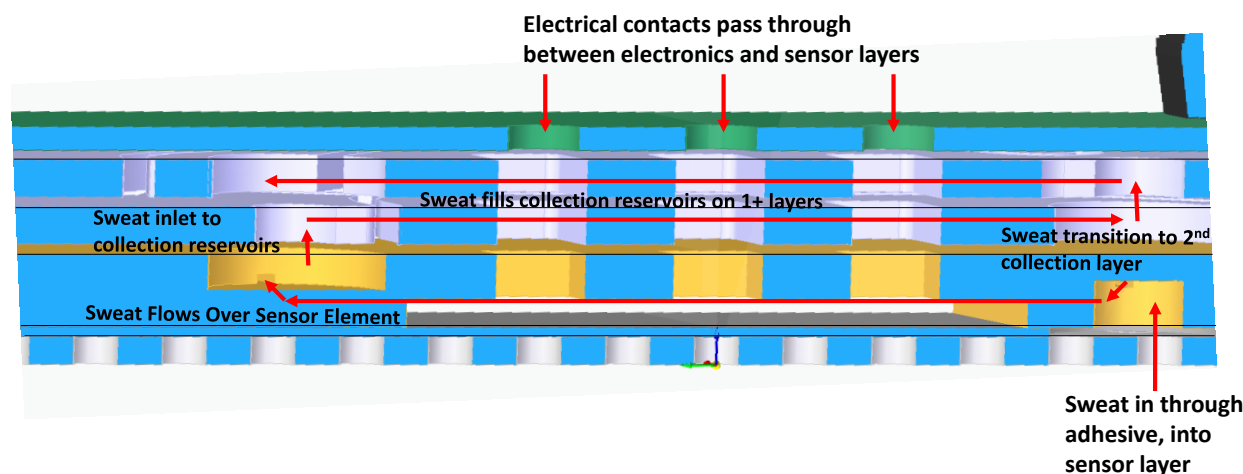


Figure 20: Cross-section view of initial prototype design showing sweat flow path

Joining of the various layers is expected to leverage adhesives and conventional microfluidic techniques. Integration of the adhesive/aggregation layer (e.g. 3M medical grade products) and the microfluidic (i.e. PDMS) sensor layer would be accomplished through the use of an adhesive coupled with oxygen plasma activation of the PDMS surface and thermal annealing. Microfluidic layers made from PDMS would be integrated together using

oxygen plasma activation and bonding. The electronics layer would be fabricated on polyimide (e.g. Kapton) to preserve device flexibility and to minimize thickness. Bonding of this layer to the underlying microfluidic (PDMS) layer could be accomplished through oxygen plasma bonding or through an adhesive plus thermal annealing.

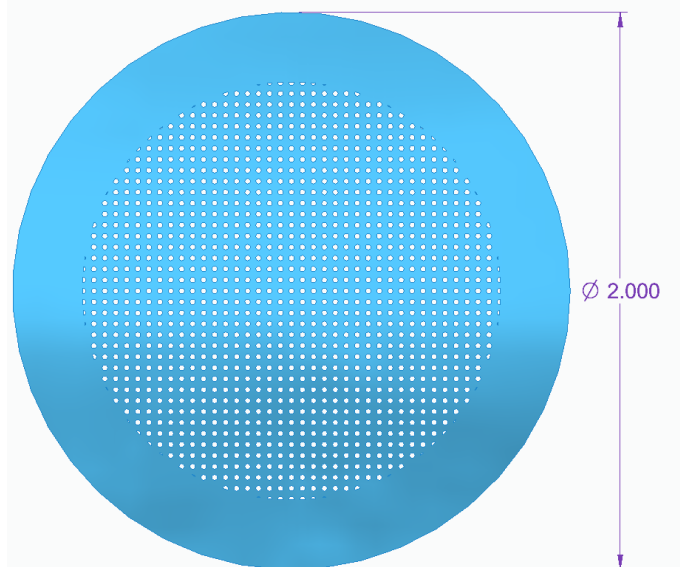


Figure 21: Adhesive layer with perimeter sealing to skin and device

The adhesive layer shown in Figure 21 is a design building upon existing, commercially available medical grade adhesive. During Task 1, CRG demonstrated the feasibility of sweat transport across a porous, breathable medical grade adhesive layer (3M 9917). Those demonstrations utilized a globally porous layer which would allow for sweat to leak from edges when/if the layer were to become sweat saturated, decreasing the driving force for continued sweat flow through the modular biosensor patch. The present adhesive layer design would leverage a base non-porous commercial, medical grade adhesive tape (e.g. 3M 1509, 1510, or 1577) which would be patterned with perforations (e.g. by LASER or mechanical punching) to create a central porous region and a nonporous perimeter annulus. The perimeter annulus would force sweat flow through the device as well as enhancing the strength of the bond to skin. Changing to a perforated – but otherwise nonporous – adhesive layer would also allow for some thickness reduction (< 4 mil) relative to the breathable film (12 mil) explored during this Phase I effort.

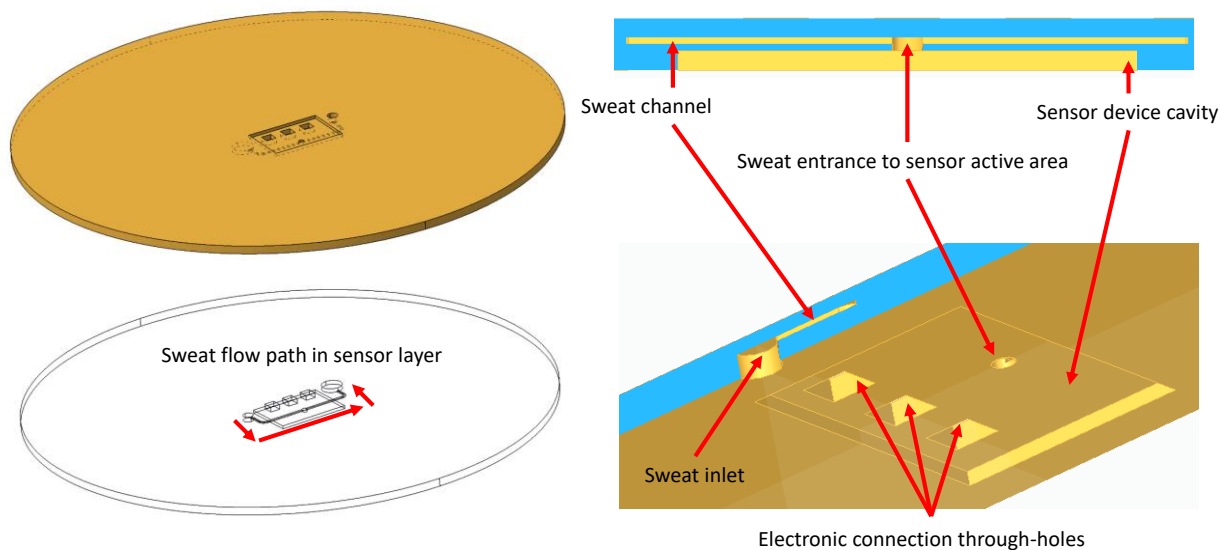


Figure 22: Sweat analysis sensor layer: isometric and wireframe views (left) and detail views(right)

The sensor layer shown in Figure 22 is a design intended for supporting modularity with respect to sweat analysis sensors. The layer consists of a pocket into which the sensor is enclosed, and simple microfluidic channel directing the flow of sweat to the active sensing area. Windows are available for passing electrical connections from the sensor to the measurement electronics layer. By leaving this layer simple, it could be modified to accommodate the dimensions of different sensors. Alternatively, if sensors could be forced to conform to a fixed footprint and fixed locations of electrical connections, there could be area not needed for sensing which could be repurposed toward other uses, such as integration of additional sensors or additional sweat collection. As currently designed, this layer is relatively thick at 31 mil ($\sim 790\text{ }\mu\text{m}$). This thickness was driven by the glass substrate used with the CRG OFET from measurement demonstration described above. It is likely that a different, much thinner, substrate such as Kapton, will be used to reduce this layer to $\sim 400\text{ }\mu\text{m}$.

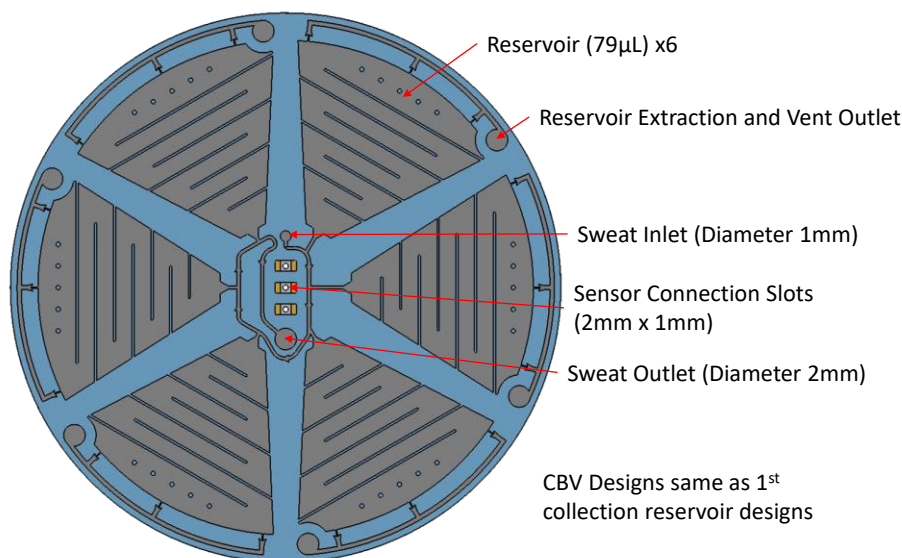


Figure 23: Sweat reservoir layer prototype design with capability for multiple layer replication

The sweat collection/reservoir layer shown in Figure 23 is a design incorporating the features demonstrated during this program, including CBVs and serpentine flow pattern within a reservoir. Additionally, this design incorporates pass through windows for electrical connections between sensor and electronics. Lastly, thought was put into the layout to enable stacking of multiple instances of the same layer by 180° rotation in order to increase sweat storage capacity. As shown, the layer is $500\text{ }\mu\text{m}$ thick with a feature depth of $400\text{ }\mu\text{m}$. The resulting reservoir capacity is $\sim 79\text{ }\mu\text{L}$ per reservoir for a total of $\sim 475\text{ }\mu\text{L}$ for the layer. Some areas of the design have been left thicker between reservoirs to help with layer robustness, but some or most of this “spoke” area could be reapportioned to the reservoirs to increase capacity.

Schematic and initial physical layout designs for the measurement circuit are shown in Figure 24 and Figure 25. These two figure represent first the fundamental interconnections enabling the operation of the measurement circuit and second a demonstration of the full collection of circuit components notionally positioned within the target envelope of the modular sweat sensor patch. Full physical routing within the circuit substrate has not been done, but this would be needed to merge the schematic and layout aspects of the design.

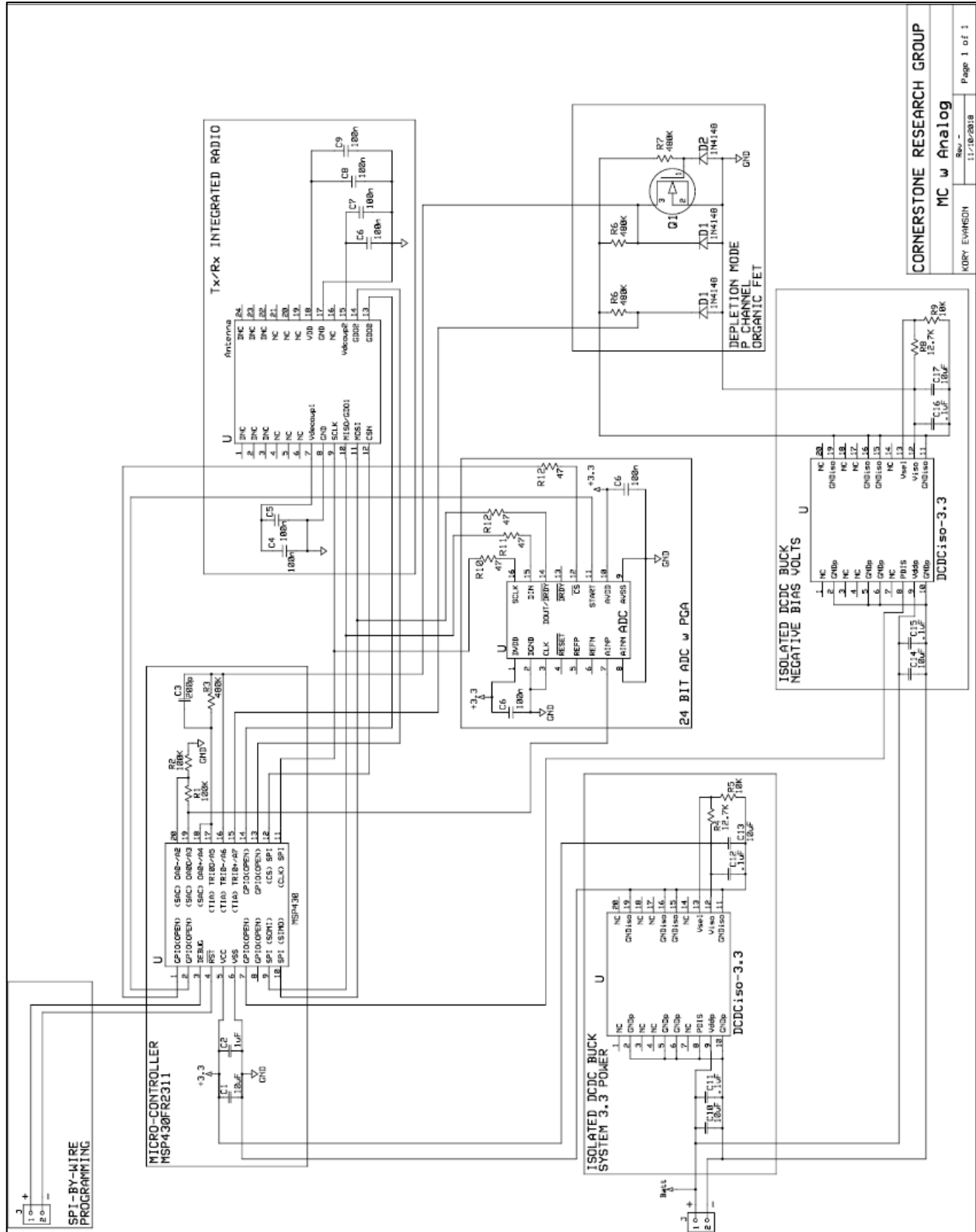


Figure 24: Measurement circuit schematic design

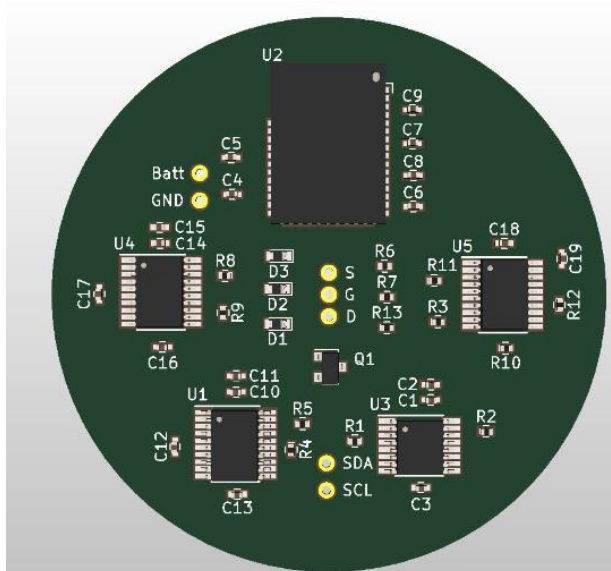


Figure 25: Initial measurement circuit component layout

Figure 26 shows a rendering of the modular biosensor patch with an attached battery pack. This battery pack would consist of one or more thin, flexible primary batteries (e.g. BrightVolt) stacked in parallel for increased capacity as needed and sealed in a flexible coating. The leads from this battery pack would be connected to the modular biosensor patch to activate the electronics at the time of use. The battery pack would also utilize medical grade adhesives to attach to the skin near to the patch device. Examples of device placement are shown in Figure 27. Even with this two part patch and battery configuration, the impact to the wearer is small.

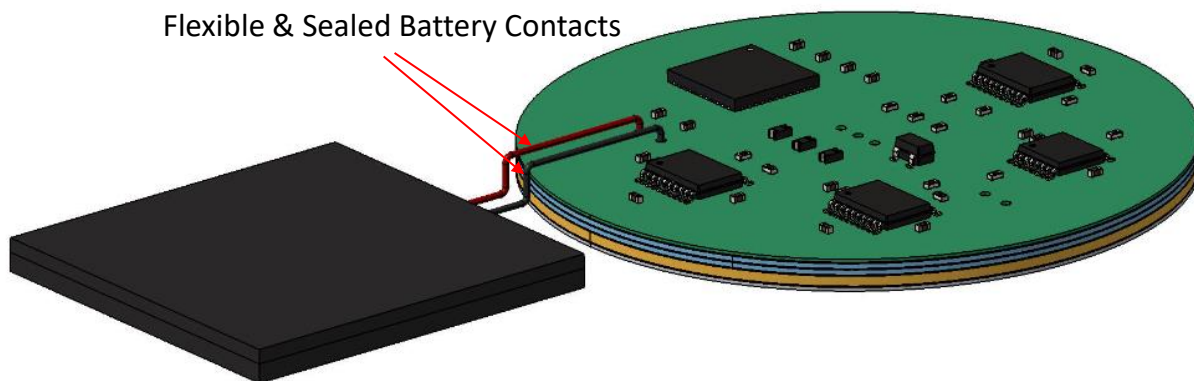


Figure 26: Rendering of modular biosensor patch and umbilical power/battery supply

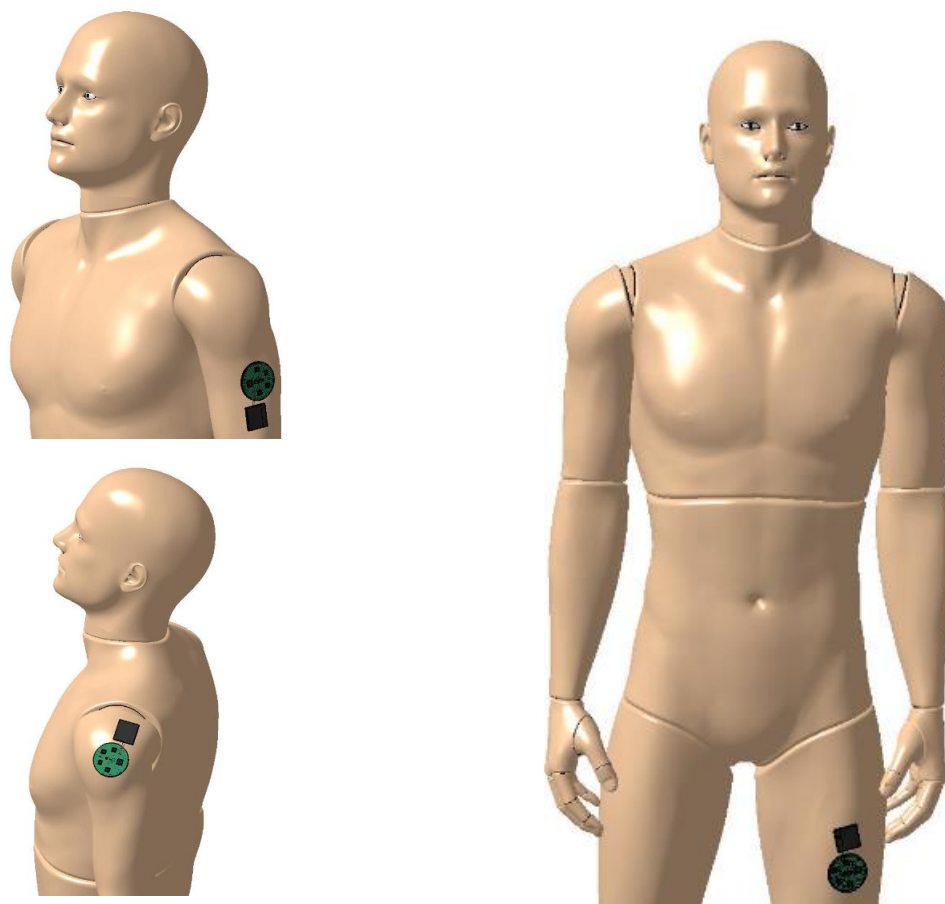


Figure 27: Examples of patch locations on a model.

Task 4 Assess results and prepare for Phase II

CRG performed a final assessment of the results and developed a Phase II plan for advancing the maturity of the Modular Biosensor Patch to provide non-invasive, continuous monitoring of sweat based biomarkers. CRG believes the Modular Biosensor Patch has achieved a TRL 3 with partial TRL 4 metrics demonstrated. Phase I objectives were satisfied as described below:

- To select components with scalable and cost effective manufacturing
 - COTS components were identified for achieving a low signal capable and low power measurement circuit, including RF transmission of data.
 - Sweat collection microfluidics were fabricated using readily available manufacturing techniques. Further design refinement/optimization may push for more aggressive design rules.
 - Device layer integration is anticipated to follow conventional approaches for PDMS based microfluidics including oxygen plasma surface activation, the use of adhesives, and thermal annealing.
- To develop a chronological sweat collection fluidic
 - Passive flow control was identified as most viable for an untethered, wearable device, leading to the selection of capillary burst valves as the critical microfluidic component.
 - A mechanical carousel was found to be impractical for a wearable device due to power requirements, low tolerance for vibration, and low ability to align to fluidic channel(s) for sweat collection. A passive array of reservoirs was pursued in its place.
 - Hundreds of microliters of sweat could be collected with the currently developed microfluidic storage approach. Duration of collection is dependent upon actual sweat rates.

- Sweat could be guided to a microfluidic device inlet from larger sampling area through the relatively simple method of a porous hydrophilic medium such as filter paper. *
- Sweat analysis could be achieved in a (semi)continuous manner with analysis occurring before storage. *
- Leak-free sweat collection will depend upon use of sufficiently strong medical grade adhesives for application of the device to skin as well as upon strong bonding between device layers. *
- To develop fluidic compatible, flexible sensor electronics platform *
- Biosensor signals range from submicroampere levels to millivolt levels depending on the style of sensor and analyte. The electronics platform should be capable of measuring such low signals with good resolution. *
- Multi-capability integrated microcontrollers are important for achieving low power demand measurement electronics. *
- Low level signal measurement necessitates 24-bit analog-to-digital converter resolution because amplification of signals for use with 10-bit ADC produces high noise-to-signal ratio. *
- Power demands 1.4 μ Ah to perform a wake-measure-transmit cycle. Device runtime therefore depends on the combination of measurement interval and available power pack size. Measurement intervals of 10-60 s could be achieved with 1-3 thin film batteries in parallel (17-25 mAh each). *
- To develop an integrated device design *
- A feasible sweat analysis and collection device could be designed within the target Phase II dimensional envelope. Phase I design with multiple reservoir layers is only slightly too thick. *
- Assembly of the multilayer device stack is expected to utilize standard PDMS joining using oxygen plasma activation and thermal annealing. *
- Scaled manufacturing methods are widely applicable for the adhesive and electronics layers. Microfluidic layers utilize normal processes, enabling the use of scale manufacturing approaches available for microfluidic production. *

3 KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated capillary burst valve control over reservoirs with 100 μ L individual volume.
- Demonstrated breadboard level circuit for measurement of low current (nA to μ A) signals such as those from OFET biological sensors.

4 CONCLUSION

During this effort, CRG demonstrated feasibility of subsystems needed to realize a complete chronological sweat analysis and collection device. This device when fully realized would enable continuous in operando monitoring of subject sweat for biomarkers of interest in a small, unobtrusive package.

Sweat control including aggregation, transition to a microfluidic system, and sequential filling of reservoirs was demonstrated at the bench level using synthetic eccrine sweat driven by a syringe pump. Design of the microfluidics included investigation of multiple valve/control mechanisms before selection of capillary burst valves (CBV) as the primary flow control element. CBVs enable flow control without the need for external drivers such as pneumatic pressure. Evaluation microfluidic devices were fabricated from polydimethylsiloxane (PDMS) for its flexibility and compatibility with aqueous systems like sweat and human skin contact. Sequential filling was demonstrated over six approximately 100 μ L reservoirs and evaluated multiple reservoir designs to target reliable and full filling. Sweat aggregation through a porous/breathable adhesive layer to an outlet point was demonstrated as a subsystem as well as with partial integration to a simple microfluidic channel to show transitions from sweat source, through an adhesive layer, and into the microfluidic sweat sensing and collection device.

An electronic circuit was designed and demonstrated for measurement of low level signals and integration as part of the sweat sensing and collection device. The circuit using a 24-bit analog to digital converter (ADC) was demonstrated in a breadboard configuration to resolve currents in microampere to nanoampere ranges, suitable for advanced sensors such as organic field effect transistors (OFET) as well as other types of sensors including electrochemical, potentiostatic, and resistive. The electronic measurement circuit demonstrated the ability to detect a sub-microamp signal from an OFET device when exposed to synthetic eccrine sweat.

Design efforts for an initial prototype design were conducted, laying the basis for Phase II refinements towards meeting Phase II objectives and fabrication of a fully functioning prototype device. Phase II effort would focus on refinement of microfluidic design rules to maximize sweat storage volume, implementation of sweat rate measurements, integration of a prototype device (microfluidics, sensor, and measurement circuit), and performance characterization with synthetic sweat.

5 PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

None

6 INVENTIONS, PATENTS, AND LICENSES

None

7 REPORTABLE OUTCOMES

None

8 OTHER ACHIEVEMENTS

None

9 REFERENCES

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10 APPENDICES

None

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