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# Non-Invasive Sweat-Based Tracking of L-Dopa Pharmacokinetic Profiles Following an Oral Tablet Administration

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**Abstract:** *Levodopa (L-Dopa) is the "gold-standard" medication for symptomatic therapy of Parkinson disease (PD). However, L-Dopa long-term use is associated with the development of motor and non-motor complications, primarily due to its fluctuating plasma levels in combination with the disease progression. Herein, we present the first example of individualized therapeutic drug monitoring for subjects upon intake of standard L-Dopa oral pill, centered on dynamic tracking of the drug concentration in naturally secreted fingertip sweat. The touch-based non-invasive detection method relies on instantaneous collection of fingertip sweat on a highly permeable hydrogel that transports the sweat to a biocatalytic tyrosinase-modified electrode, where sweat L-Dopa is measured by reduction of the dopaquinone enzymatic product. Personalized dose-response relationship is demonstrated within a group of human subjects, along with close pharmacokinetic correlation between the finger touch-based fingertip sweat and capillary blood samples.*

Parkinson disease (PD) is a chronic, progressive neurodegenerative disease affecting more than 6 million individuals worldwide.[1,2] Levodopa (L-Dopa), the precursor of dopamine, is the most effective drug for symptomatic management of PD and is considered the gold standard treatment.[3,4] However, due to progressive neuronal death from PD and L-Dopa's short half-life, its long-term administration is associated with the onset of motor and non-motor complications, stemming mainly from fluctuations in the plasma L-Dopa level.[5] Over time, L-Dopa's therapeutic window narrows, resulting in fluctuating symptoms; suboptimal dosing causes PD patients to experience muscular stiffness (rigidity), slowed movements (bradykinesia), and tremors, while overdosing generates excessive, involuntary movements (dyskinesias).[6] The therapeutic window becomes narrower with disease progression, which requires patients to take higher doses at more frequent intervals. Further complicating the treatment of PD, there is a high interpatient variability in the response to L-Dopa therapy, which necessitates a fine-tuned, personalized dosing regimen based on each individual patient's characteristics and lifestyle.[7] Such inconsistent dramatic fluctuations in the plasma drug concentrations hamper the management of PD patients, and leads to suboptimal therapeutic efficacy, particularly during disease progression.[8–10] Therefore, a device capable of rapidly monitoring L-Dopa levels would offer attractive advantages toward precise and personalized L-Dopa dosing, thus avoiding motor and non-motor complications in PD patients. Currently, no commercial devices exist that are capable of real-time monitoring of L-Dopa levels and the corresponding pharmacokinetic profiles.

The current "gold standard" method to detect plasma L-Dopa levels relies on liquid chromatography-mass spectroscopy (LC-MS), which is performed in centralized laboratories.[11] Due to this technique's invasiveness (blood draws), long turn-around times (days), and the need for specialized instruments and skilled personnel, these measurements cannot support personalized PD treatment and related timely corrective therapeutic action. Thus, decentralized mobile and wearable electrochemical sensors, in the form of disposable enzyme strips,[12] microneedles,[13] and sweat band,[14] have been proposed for frequent and continuous L-Dopa monitoring. However, such sensors have been confined primarily to in vitro demonstrations. A single in vivo study, involving an epidermal band has been reported in connection to uptake of fava beans, and not the standard pill formulations, making its relevance for therapeutic monitoring of PD patients unclear.

Here, we present the first demonstration of individualized therapeutic drug monitoring, based on dynamic, non-invasive tracking of sweat L-Dopa levels upon administration of standard L-Dopa pill formulations based on a single fingertip touch. Sweat is a non-invasively retrievable biofluid containing rich information of health-related biomarkers.[15,16] Wearable sweat sensors have recently shown enormous potential toward non-invasive monitoring of physiological health status (e.g., hydration[17]) and disease diagnosis and management (e.g., diabetes,[18] gout,[19] and pain[20]). However, accessing the information-rich sweat biofluid commonly requires perspiration assistance (involving physical exercise and electrical or thermal stimulation) which hinders practical drug monitoring applications. Unlike these vigorous sweat stimulation methods, natural perspiration at the fingertip has recently demon-

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strated immense potential for convenient sweat sampling for chemical analysis.[21] Taking advantage of the high density of eccrine sweat glands ($\approx 400$ glands cm$^{-2}$) and corresponding high sweat rates at the fingertips, touch-based biosensors have been reported recently for the simplified non-invasive detection of key sweat biomarkers, such as glucose[22] or cortisol,[23] without any sweat stimulation. In the following sections, we will demonstrate how such touch-based sweat sensing can be combined with tyrosinase-based enzyme electrodes[12] for realizing non-invasive tracking of L-Dopa pharmacokinetic profiles.

The new fingertip L-Dopa biosensor leverages such naturally secreted sweat to (semi)continuously monitor the dynamic profile of sweat L-Dopa following the administration of a single-dose of L-Dopa-Carbidopa (C-Dopa) immediate release (IR) oral tablet (100:25 mg) (Figure 1 A (a)). The

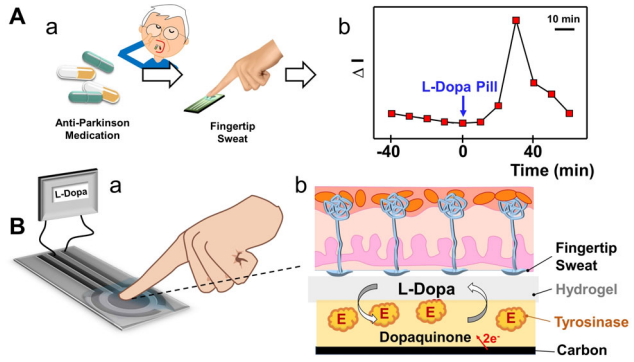


***Figure 1.*** Working principle of the fingertip L-Dopa biosensor. A. (a) The finger touch-based procedure before and after intake of the antiparkinsonian medication, L-Dopa and (b) the typical current-time profile recorded every 10 minutes B. (a) Depiction of the underlying mechanism of L-Dopa detection, starting with (a) touching the sensor with index finger, (b) transfer of natural sweat containing L-Dopa from the skin surface through the porous hydrogel to the electrode surface, where it is electrochemically measured at tyrosinase immobilized electrode.

current signal difference measured at 10-minute intervals after administering the pill shows a rapid rise in sweat L-Dopa response, increasing to its peak value 30 min after the drug intake, and then declines rapidly to its background level (Figure 1 A (b)). Interestingly, validating the dynamic sweat L-Dopa temporal profile by comparing to versus capillary blood samples showed a similar pharmacokinetic profile with a short ($\approx 10$ minutes) lag time. Such detection of sweat L-Dopa simply relies on touching with index finger a sweat-wicking porous hydrogel, placed on the printed biosensing electrode, to allow fast transfer of the biofluid to the surface of the enzyme-modified electrode strip (Figure 1 B). The immobilized tyrosinase enzyme oxidizes the sweat L-Dopa to dopaquinone, which is selectively detected using a low cathodic potential of $-0.3$ V. The resulting amperometric reduction current signal thus reflects the dynamically changing L-Dopa sweat levels following the pill intake. This non-invasive, fast, and simple touch-based protocol holds considerable promise for capturing fluctuations in the sweat L-Dopa concentrations in near real-time, toward guiding individualized treatment of PD patients, involving fine-tuned L-Dopa dosing, and improving therapeutic efficacy and PD management without frequent piercing of the finger.

L-Dopa detection was achieved through coupling the tyrosinase enzyme-catalyzed L-Dopa oxidation (catecholase activity[12,24]) and the subsequent low-potential electrochemical reduction of the corresponding quinone product, dopaquinone (Figure 2 A). The formed reaction cycle not only

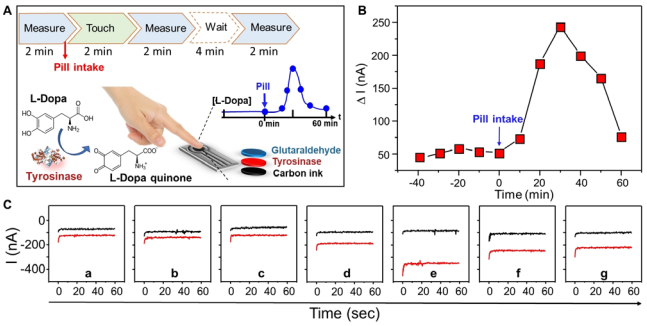


***Figure 2.*** A representative on-body experiment of L-Dopa monitoring using the touch-based sensor. A. Time course of a cycle of L-Dopa detection in fingertip sweat, consisting of measuring the current before touching (2 min), touching (2 min), measurement after touching (2 min), and waiting for the next cycle (4 min). B. Dynamic pharmacokinetic profile during intake of an L-Dopa/C-Dopa pill by the subject. C. (a–g) The corresponding chronoamperograms obtained from time $-10$ min to $+60$ min (three initial amperograms of $-40$, $-30$, and $-20$ min are not shown for clarity), where the black and red curves represent readouts before and after touching, respectively.

enhances the sensitivity through amplification of the resulting current signal but also prevents electrode fouling by inhibiting the spontaneous polymerization reactions of the unstable quinone molecules. Tyrosinase enzyme was immobilized on the surface of screen-printed carbon electrodes, followed by crosslinking with glutaraldehyde to prevent leaching of the enzyme (Figure 2 A). To establish the optimal potential for the L-Dopaquinone reduction, the amperometric signals obtained at various potentials, ranging from $-0.1$ to $-0.4$ V (vs. internal pseudo-reference Ag/AgCl), were compared upon addition of 10 μM L-Dopa (Figure S1). The current response dramatically increased upon decreasing potential from $-0.1$ to $-0.3$ V, after which the signal declined probably due to the interference from oxygen reduction reaction. Thus, the optimal potential of $-0.3$ V was chosen and used for subsequent in vitro and on-body experiments. Figure S2(A,B) shows the resulted amperometric responses of L-Dopa addition from 5 to 30 μM, giving well-defined linearity over the whole physiological concentration range. It shows also the ability to detect even 1 μM L-Dopa concentrations, with an estimated detection limit of 300 nM (inset). The reproducibility of the L-Dopa sensor was evaluated by measuring 10 μM L-Dopa concentration on six different sensors (Figure S2(B)). The results revealed high fabrication reproducibility of the sensor with a relative standard deviation (RSD) of 2.6%. Moreover, the carry-over experiment was performed to evaluate the repeatability ($n = 5$) of sensor response to alternating L-Dopa solutions of 0 and 15 μM,


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showing negligible signal drift (Figure S2(C)). The sensors, kept at 4 °C, showed high storage stability, retaining 97.3 % of initial response after one week (Figure S3).

The performance of the sensor toward following the L-Dopa pharmacokinetics was characterized on healthy volunteers following the administration of one L-Dopa/C-Dopa (100:25 mg) IR oral tablet, which is the conventional and standard dose of oral medication for PD patients. C-Dopa is an amino acid (dopa) decarboxylase enzyme inhibitor and is combined commonly with L-Dopa to enhance the bioavailability of the drug. C-Dopa is an o-diphenolic compound that can also be oxidized by the tyrosinase enzyme, and thus may interfere with the detection of the target L-Dopa. The selectivity of the sensor against C-Dopa, examined by detecting L-Dopa/C-Dopa in 4:1 concentration ratio (the same composition as the tablet), showed negligible C-Dopa interference of around 5 % (Figure S4). Interestingly, even the presence of equal C-Dopa concentration resulted in a small (20 %) interference. These data indicate minimal interference of coexisting C-Dopa molecules, as desired for accurate and reliable detection of the L-Dopa target. The effects of other common interfering molecules (i.e., ascorbic acid and uric acid), common stimulants (i.e., caffeine), and other constituents that can act as substrates for tyrosinase (e.g., acetaminophen, tyrosine, dopamine, resorcinol) were also investigated in the maximum possible bodily level for each compound. The results, shown in Figure S5, clearly confirmed the high selectivity of the sensor toward L-Dopa detection. Typical measurements of the target L-Dopa following the pill intake have been carried out at 10-minute intervals. Figure 2 A depicts the optimal time course of a single 10-minute cycle of the sweat L-Dopa sensing protocol, including an initial 2-minute recording of the background current in a buffer solution-soaked porous hydrogel coated electrode (without fingertip touch), followed by placing the index finger on the gel (covering the working electrode) for 2 minute, during which sweat diffuses to the electrode surface, and subsequently stepping the potential to −0.3 V recording the current signal for 2 minutes. Following each cycle, the subject was asked to wait for 4 minutes before starting the next cycle. The current difference between before and after touch was plotted vs. time to correct for any non-drug-related current fluctuations and thus, to ensure that changes in the signal reflect solely the uptake and metabolism of L-Dopa. Figure 2 B displays a typical peak-shaped dynamic profile of the subject sweat L-Dopa levels over the 100-minute test period, involving 5 and 6 measurements before and after taking the pill, respectively. The corresponding raw current signals and backgrounds are shown in Figure 2 C. These data of Figure 2 B and 2 C show negligible current changes prior to the pill intake, with the L-Dopa current signal starting to increase 10 minutes after the pill intake. The signal reaches its maximum peak at 30 minutes, after which it decreases back to its background level nearly one hour after taking the pill. These results clearly demonstrate that the touch-based L-Dopa sensor can successfully track variation of L-Dopa sweat levels. Analogous blood L-Dopa strips would require frequent skin piercings to record similar temporal profiles.[12] As illustrated below, such peak-shaped temporal sweat profile matches closely with the pharmacodynamics of the corresponding blood L-Dopa concentration (with a short ≈ 10-minute time delay).

To gain further insight into the personalized subject responses upon medication intake, the performance of the finger touch biosensor was evaluated on three different healthy individuals while taking the same medication under identical conditions (Figure 3 A a–c). The subjects were asked

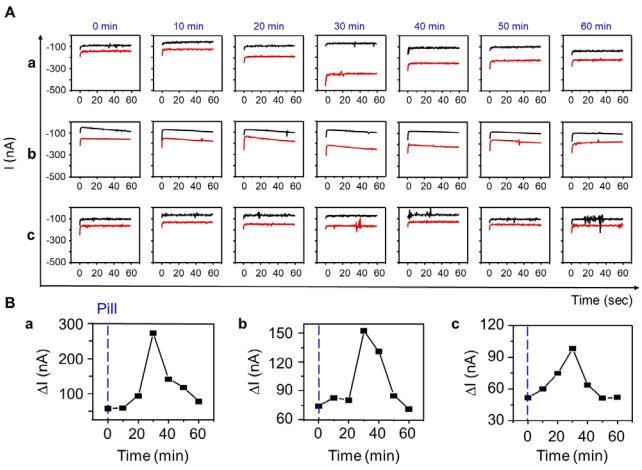


***Figure 3.*** Personalized pharmacokinetic profiles of L-Dopa drug in three different healthy subjects. A. Chronoamperograms obtained every 10 min for three subjects (a–c), with black and red curves showing before and after touching current measurements. B. The relevant temporal current profiles for three subjects. The blue dotted line in each diagram indicates the time of pill intake (0 minute).

not to eat any food or drink caffeinated liquids for two hours before the experiment to exclude any unwanted potential interference from dietary proteins or from caffeine. The same protocol used in Figure 2 A was followed for the sweat collection and signal recording in each subject. Interestingly, all three subjects display relatively similar temporal profile of sweat L-Dopa level, reaching their peak maximum 30 minutes after pill intake. The slightly different profiles are attributed to interindividual pharmacokinetic variability in the response to L-Dopa. Note, also the largely different peak currents observed for the three subjects, with the highest and lowest signals obtained for the first (a) and third (c) individuals, respectively. These variations reflect primarily differences in the sweat secretion rates of the subjects, which can be addressed via a short personal calibration as was demonstrated recently for analogous sweat glucose measurements.[22] Such protocol involves a simple one-time personal pre-calibration to create personalized algorithm to account for the individual properties.

To further confirm the reliability of the new touch-based sweat L-Dopa detection, the feasibility of data verification between sweat and blood samples was investigated. Two subjects performed fingertip sweat sensing in parallel to the electrochemical measurements of the finger pricked capillary blood samples, using a similar tyrosinase-modified L-Dopa sensor. As illustrated in Figure 4 A and 4 B, the sweat fingertip sensor was able to capture dynamic profile of the blood L-

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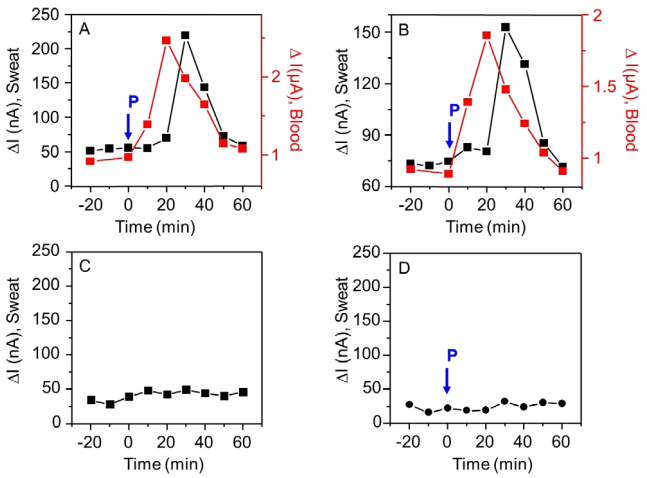


***Figure 4.*** Pharmacokinetic correlation of response to L-Dopa using natural sweat and capillary blood samples. A,B. Continuous monitoring of sweat (black) and blood (red) L-Dopa every 10 min in different subjects. C,D. The results of control experiments performed without pill consumption (C) and using electrode without enzyme modification (D). The letter "P" indicates the time of pill intake.

Dopa measurements, with ≈10 minutes of lag time. The blood L-Dopa level started to increase 10 minutes after taking the pill, reaching its peak concentration within 20 min. The sweat L-Dopa was detectable more than 20 min after the pill intake, with a peak concentration time of 30 min. Such similar blood and sweat L-Dopa profiles result in Pearson's correlations (Pr) of 0.94 and 0.87 for subject 1 and subject 2, respectively (Figure S6). Introducing time-lag correction would enable the blood L-Dopa profile to be reconstructed in real-time from the continuous sweat measurements, analogous to similar lag corrections reported for monitoring glucose.[25] Note that while such blood profiling would require eight repeated skin piercings within 80 minutes, the sweat L-Dopa tracking involves only multiple fingertip touches, hence simplifying greatly the drug profiling. Control experiments were also performed without a pill intake (Figure 4C) and using a non-enzymatic electrode (Figure 4D). In both control experiments, the current signal remained very small and unchanged (compared to the large peak currents of A and B), indicating that the biosensor response is due to the pill administration and to the tyrosinase L-Dopa enzymatic reaction. Overall, the data of Figure 4 and Figure S6 demonstrate the ability to monitor non-invasively in near real-time individualized pharmacokinetics after oral L-Dopa intakes while obtaining good correlation to parallel blood fingertip measurements. Such first demonstration of pharmacokinetic correlation for sweat and blood L-Dopa profiles suggests considerable potential of the touch-based sweat protocol for non-invasive tracking of L-Dopa's the dynamic pharmacokinetic action in PD patients.

In conclusion, we have demonstrated, for the first time, that non-invasive sweat measurements offer considerable potential for tracking the pharmacokinetic profiles of L-Dopa following a single-dose oral tablet administration. This sweat-based L-Dopa analysis was realized by combining simple and rapid sampling of natural perspiration accumulated on one's fingertips with highly sensitive and selective tyrosinase enzyme-based electrochemical assay. The ability of the new sweat-based strategy to monitor individualized pharmacokinetics after oral L-Dopa intakes in near real-time was demonstrated successfully in a small group of healthy subjects by recording the amperometric response in 10-minute intervals. The close pharmacokinetic correlations of the fingertip sweat and blood profiles confirmed the reliability of this method. Future efforts will include extensive clinical evaluations with large population of PD patients along with parallel data validation using the gold-standard LC-MS analysis. Overall, such dynamic non-invasive profiling of L-Dopa pharmacokinetics offers a tremendous potential for addressing current challenges of treating PD symptoms associated with the narrow L-Dopa therapeutic range and high inter-patient variability, thus providing a step forward towards personalized treatment of PD. The touch-based non-invasive fingertip sweat therapeutic monitoring concept can be expanded to other important drugs and will open new possibilities toward personalized pharmacotherapies and optimal management of other diseases. Other biorecognition routes, particularly the use of aptamers,[24] should also be considered toward potential in vivo L-Dopa monitoring.

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## Conflict of Interest

The authors declare no conflict of interest.

**Keywords:** biosensor · Levodopa · Parkinson disease · phamacokinetics · therapeutic drug monitoring

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# Communications

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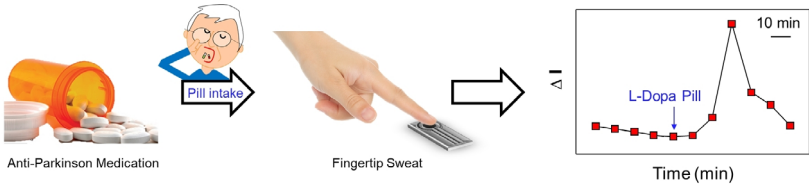


Non-invasive touch-based sweat measurements are used for tracking the pharmacokinetic profiles of levodopafollowing a single-dose oral tablet administration.


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