Abstract

Development of a Wearable Sweat Sensor Chip Based on Surface-Enhanced Raman Spectroscopy †

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Abstract: A wearable sweat sensor chip based on surface-enhanced Raman spectroscopy is developed. The plasmonic core of the chip, obtained by depositing silver nanowires on a PTFE porous membrane, permits the direct and label-free detection of urea and lactate at physiological concentration in combination with the evaluation of sweat pH in the range between 4 and 9.

Keywords: wearable sensor; SERS; sweat; urea; lactate; pH; silver nanowires

1. Introduction

In recent decades, wearable sensors have received much attention thanks to their ability to provide useful information about the metabolism and health of individuals [1]. Wearable devices originally developed for tracking physical exercise activity (i.e., heart rate, calories burned, step count) or to monitor biomedical parameters (i.e., glucose concentration in blood) have prompted major technological efforts to turn them into advanced biosensing platforms (lab-on-chip) for the continuous monitoring of multiple health parameters and real-time sensing of physiological information with a potentially large impact in our daily lives [1,2]. Sweat contains a broad variety of biomarkers (electrolytes, proteins, metabolites, etc.) and can provide access to chemical information directly related to body health in a non-invasively accessible, continuous and on-demand way. Moreover, compared with other biofluids like blood, interstitial fluid, tears, saliva, and urine, sweat sampling can be easily achieved by placing a sensor patch on accessible locations of the skin [2].

Surface-enhanced Raman scattering (SERS) is a spectroscopic technique that permits the label-free detection and vibrational characterization of analytes at sub-micromolar concentrations thanks to the plasmonic amplification of the Raman optical signals generated in proximity to noble metal nanoparticle surfaces (hot spots) [3,4]. The possibility of easily integrating SERS-active layers onto flexible substrates, together with the recent development and commercial availability of low-cost, miniaturized, and handheld Raman spectrometers, has paved the way for the development of optical technological platforms for non-invasive healthcare monitoring via sweat inspection.

In this work, we resume our effort in the development of SERS-active chips based on silver nanowires (AgNWs) deposited on a flexible PTFE-membrane substrate. We integrated the as-fabricated AgNWs@PTFE with biocompatible and protective adhesive layers to obtain a fully flexible and wearable sensor chip for the detection of analytes from sweat samples.

2. Materials and Methods

AgNWs@PTFE chips were produced following the method recently reported by our group [4,5] and their ability in the detection of urea and lactate from an artificial sweat solution was tested. In parallel, identical chips were integrated in a biocompatible tape sandwich including Tegaderm (3MTM) and Leukomed (Leukoplast) as protective/adhesive and spacer layers, respectively.

A simulated sweating system combining a syringe pump, which injects the sweat sample at a constant rate, a 3D printed transparent cell (Clear Resin Formlabs), and a microporous polyimide foil which mimics artificial skin was developed and used to test chip performances against an artificial sweat solution made of 0.1% urea, 0.1% lactate, and 0.5% NaCl in ultrapure water (chemicals purchased by Merck).

A pH-sensitive probe molecule (4-mercaptobenzoic acid; 4-MBA) was employed to functionalize AgNWs and evaluate the pH levels of sweat solutions [6]. Experiments on real sweat samples were performed directly by sticking the biocompatible SERS chip onto volunteers' arms and collecting the secretion during physical exercise.

SERS spectra were acquired using a micro-Raman spectrometer (LabRam, Horiba, France) equipped with an excitation laser tuned at 785 nm, focused through a $50\times$ LWD objective (Olympus, Germany), and using a laser power at a sample and integration time fixed at 400 μW and 5 s, respectively.

3. Discussion

Our laser-patterned spot-deposition method, recently described [4,5], guarantees spatial control over the AgNWs area on PTFE, producing a confined SERS-active hydrophilic region surrounded by a hydrophobic one. The immediate advantage is that a few tens of μL of sweat secreted by the skin are enough to flow through the PTFE pores and are forced to be adsorbed on the AgNWs surface without the need for microfluidic channels. We demonstrated the ability of the chip in the SERS detection of urea and lactate in artificial sweat at concentration values ranging between 0.01% and 1.5% (w/v), observing a linear trend in the analyzed interval. Similarly, upon functionalization with 4-MBA of AgNWs, we were able to measure the pH of sweat as the variation of the ratio between the 1400 (carboxylate stretching [7]) and the 1590 cm-1 (aromatic ring breathing [7]) Raman peaks related to the deprotonation of 4-MBA molecules at increasing pH. A final validation of the chip ability in the detection and quantification of urea and lactate sweat content from a volunteer during physical exercise was demonstrated.

Institutional Review Board Statement: The experimental procedures involving volunteer human subjects described in this research follow the ethical principles outlined in the Helsinki Declaration of 1964, revised in 2013. No ethical approval is necessary at this project stage.

Informed Consent Statement: The participant gave their informed consent for inclusion before participating in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kim, J.; Campbell, A.S.; de Ávila, B.E.F.; Wang, J. Wearable biosensors for healthcare monitoring. *Nat. Biotechnol.* **2019**, *37*, 389. [CrossRef]
2. Min, J.; Tu, J.; Xu, C.; Lukas, H.; Shin, S.; Yang, Y.; Solomon, S.A.; Mukasa, D.; Gao, W. Skin-Interfaced Wearable Sweat Sensors for Precision Medicine. *Chem. Rev.* **2023**, *123*, 5049–5138. [CrossRef]
3. Polykretis, P.; Banchelli, M.; D'Andrea, C.; de Angelis, M.; Matteini, P. Raman Spectroscopy Techniques for the Investigation and Diagnosis of Alzheimer's Disease. *Front. Biosci.* **2022**, *14*, 22. [CrossRef] [PubMed]
4. Barucci, A.; D'Andrea, C.; Farnesi, E.; Banchelli, M.; Amicucci, C.; de Angelis, M.; Hwang, B.; Matteini, P. Label-free SERS detection of proteins based on machine learning classification of chemo-structural determinants. *Analyst* **2021**, *146*, 674. [CrossRef] [PubMed]
5. Banchelli, M.; Amicucci, C.; Ruggiero, E.; D'Andrea, C.; Cottat, M.; Ciofini, D.; Osticioli, I.; Ghini, G.; Siano, S.; Pini, R.; et al. Spot-on SERS Detection of Biomolecules with Laser- Patterned Dot Arrays of Assembled Silver Nanowires. *ChemNanoMat* **2019**, *5*, 1036–1043. [CrossRef]
6. He, X.; Fan, C.; Luo, Y.; Xu, T.; Zhang, X. Flexible microfluidic nanoplasmonic sensors for refreshable and portable recognition of sweat biochemical fingerprint. *Npj Flex. Electron.* **2022**, *6*, 60. [CrossRef]
7. Chung, M.; Skinner, W.H.; Robert, C.; Campbell, C.J.; Rossi, R.M.; Koutsos, V.; Radacsi, N. Fabrication of a Wearable Flexible Sweat pH Sensor Based on SERS- Active Au/TPU Electrospun Nanofibers. *ACS Appl. Mater. Interfaces* **2021**, *13*, 51504–51518. [CrossRef]