Available online at www.sciencedirect.com

ScienceDirect

Procedia Engineering 120 (2015) 756 – 759

Procedia Engineering

www.elsevier.com/locate/procedia

EUROSENSORS 2015

Skin microbiota monitoring by Nanowire MOS Sensors.

Veronica Sberveglieri [a]* , Estefania Núñez Carmona[a,b] , Andrea Ponzoni[a] , Elisabetta Comini[a,c], Vardan Galstyan[a] , Dario Zappa[a,c] , Andrea Pulvirenti .

[a] *CNR-INO Sensor Lab,Via Valotti 9, 25133 Brescia, Italy.*
[b] *University of Modena and Reggio Emilia, Department of Live Science, 42124 Reggio Emilia, Italy.*
[c] *University of Brescia, Department of Information Engineering, Via Branze 45, 25123 Brescia, Italy.*

Abstract

In this work is illustrated the potentiality use of a Nanowire (Nw) MOS sensor array to be use as tool to monitor the human skin microbiota in order to identify quantitative and qualitative changes. Tree different blends of microorganisms grown in artificial sweat have been tested. Classical techniques, like GC-MS with SPME, have been performed as well, in order to have a multidisciplinary approach and consistent data set. The obtained results show the ability of the NW technology to discriminate between the different blends of microorganisms and to follow up the development of the growth inside the blends during the analysis. The previous results have been confirmed by GC-MS with SPME.

Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

Keywords: Nanowire MOS gas sensor; skin microbiota; artificial sweat; GC-MS with SPME.

1. Introduction

The human body can be considered as a super organism since for each one of the animal cells, 10 microorganisms are living in the skin and the mucosa. The normal microbiota (NM) is composed by fungi, yeast and in a major part for bacteria and its quantitative and qualitative composition may change due to external factors or different physiological condition [1]. The microbial antagonism is a property, which enables one microorganism to kill, or inhibit the growth of a different one. Metal Oxide Nanowires were synthetized using a simple and scalable evaporation-condensation technique, directly on final transducers used for the fabrication of the sensing devices [2].

* Veronica Sberveglieri. Tel.: +39 0522 522061.
E-mail address: veronica.sberveglieri@ino.it

Peer-review under responsibility of the organizing committee of EUROSENSORS 2015
doi:10.1016/j.proeng.2015.08.798

S3 device equipped with nanowire (Nw) technology is created for the analysis of the headspace. The features that S3 possess make possible to reveal the presence of the microorganisms trough the detection of the organic volatile compounds (VOCs) produced during their metabolic activities. The aim of this work is to set up a new approach based on the collaboration between Nw technology and classical techniques, to establish a new fouling to monitor the development of NM of the skin. Nw technology has already proven its ability to identify different single microorganism and follow up their development inside a microbial cultures [3].

2. Materials & Methods

2.1. Sample preparation

All strains have been selected from the internal collection of the Life Science Department of the University of Modena and Reggio Emilia. The cultures were incubated at 30º C during 48 h. Subsequently the cultures were centrifuged at 3000 rpm during 5 min, the supernatant was discarded and the cells were re-suspended in 3 ml of physiological solution.. At the end of the process the supernatant was discarded and the pellet was suspended in 4 ml of sterilized artificial sweat. The sterilization process was carried out at 121° C during 15 min at one over pressure atmosphere in order to eliminate any microbial contamination. For each cm^2 of skin it's possible to find 10^4-10^5 CFU (Colony Forming Unit) including prokaryotes (bacteria) and eukaryotes (mould and yeast). It is also know that and adult human being has 2 m^2 of total skin surface which means that overall it carries from $2x10^8$-$2x10^9$ CFU. Besides and adult human being produce in normal conditions 400 ml of sweat by day [3]. Furthermore in microbial clinical research articles is stated that the major part of the microorganisms that lives over the human surfaces belongs to the prokaryotes group. Three different blends of microorganisms have been inoculated in artificial sweat, in the proper rate in order to mimic the normal condition of the skin and have been incubated at 36° C in the dark during 4 days. Microbial blends contains are represented in Table 1 a Control sample was performed as well just containing sterilized artificial sweat [4]. It has been decided to take the rate 3:1 between prokaryotes and eukaryotes [5,6]. Summarizing the concentration of prokaryotes $3,75x10^6$ CFU/ml and for eukaryotes $1,25x10^6$ CFU/ml for each person.

Table 1. Microbial samples.

Mix Kinds	Microbial components
Mix A	*Listeria monocytogenes*, MMCO (yeast) and MMSI (fungi).
Mix B	*Escherichia coli*, MAP10 (yeast) and FGO3 (fungi).
Mix C	*Salmonella enteritidis*, *Candida albicans* (yeast) and FGB2 (fungi).
Control	No microbial species inoculated.

2.2. S3 device

The used device in this work is the S3 (Small Sensor System). This instrument has been created in the Sensor Lab, CNR INO Brescia in Italy [1]. S3 is equipped with a thermally controlled sensor chamber of 20 ml internal volume where are placed six MOX gas sensors. Three of these sensors were prepared with the RGTO thin film technology, and the other three constructed with nanowire technology. In fact, two of the three nanowires in the array are zinc oxides sensor but with different operating temperatures and the third one is a tin oxide sensor. The nanowire sensors manifest a very high length-to-width ratio creating a 3 dimensional network exposed to the gas. In this way, the adsorption surface is increased in a huge amount enhancing the response of the instrument and decreasing the threshold. It exhibit a remarkable crystalline quality as well. This characteristics result in an enhanced performance and a long-term stability for sustained operations. The instrument was also provided with the auto-sampler headspace system HT280 (HTA srl, Brescia, Italy), supporting a 40 loading sites carousel and a shaking oven to equilibrate the sample headspace. A volume of 2 ml for every dilution was placed individually in a sterilized chromatographic vial of 20 ml (Table 1). Once inoculated all the vials were cover with an aluminum crimp, a coated PTFE/silicon septum and crimped. This operation was repeated during the 80 h of analysis (from T0 to T80). It was used synthetic

chromatographic air with a continuous flow rate of 10 ml/min to perform the sensor baseline and the recovery time was 28 min. The data analysis was run by means of Principal Component Analysis (PCA). Data were processed by EDA software, a home written software developed in MATLAB® at Sensor Laboratory. The goal was to obtain a good visual separation of the clusters, not just to have high estimate accuracy. For this purpose, PCA was systematically used to judge the clusters separation.

2.3. Gas Chromatography- Mass Spectrometry with SPME

GC-MS analysis was performed using a Shimadzu Gas Chromatograph GC2010 PLUS (Kyoto, KYT, Japan) equipped with a Shimadzu single quadrupole Mass Spectrometer MSQP2010 (Kyoto, KYT, Japan) ultra and a HT280T auto sampler (HTA srl, Brescia, Italy) that allowed SPME analysis. The vials were incubated in an oven thermostatically regulated at 50ºC for 15 minutes due to create the headspace equilibrium. In order to extract the volatile compounds of the samples was used a DVB/carboxen/PDMS stable flex (50/30 µm) (Supelco Co.Bellefonte, PA, USA) SPME fiber. To provide the adsorption of volatile compounds the SPME fiber was exposed to the headspace of the vials for 15 minutes at 50°C. For desorption of the compounds the fiber was placed in the injector of the heated GC for 6 min at 200°C. Volatile organic compounds were separated using and analytical capillary column (DB-WAX capillary column, 30m x 0.25 mm x 0.25µm, Agilent Technologies, Santa Clara, CA, USA) and the carrier gas was ultrapure helium (99.99%) at a constant flow rate of 1.5 mL/min. The temperature program for the GC was performed in the following way: from 50° C for 5 min, followed of a linear gradient 2 C°/min to 100°C and held for 5 min, followed by a rise from 100°C to 240°C at 5°C/min, temperature held 5 min.

3. Results & Discussion

3.1. S3 device results

In the Figure 1 it is possible to observe the PCA score plot showing the development of the 4 kind of samples during the 20 first hours. Is evident the formation of a compact cluster composed by the samples containing the Mix B (blue dots). On the contrary Mix A, Mix C and Control are not distinguished. It indicates that microorganisms are able to develop in artificial sweat and create changes in the headspace of sample Mix B.

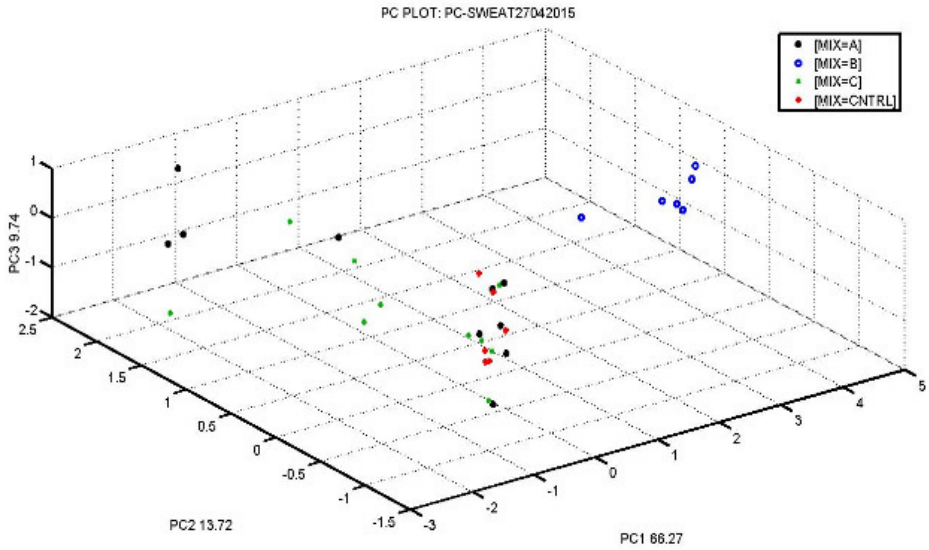

Fig. 1. PCA Score plot of the 4 samples kind in the first 20 h of analysis.

3.2. Gas Chromatography- Mass Spectrometry with SPME results

In the Figure 2 are reported the results of the GC-MS with SPME technique for the Mix B during the first 80 h of analysis. It is remarkable that the headspace is dominated by organic alcohols of short chain like butanol, and hexanol and organic acids like hexanoic acid and octanoic acid.

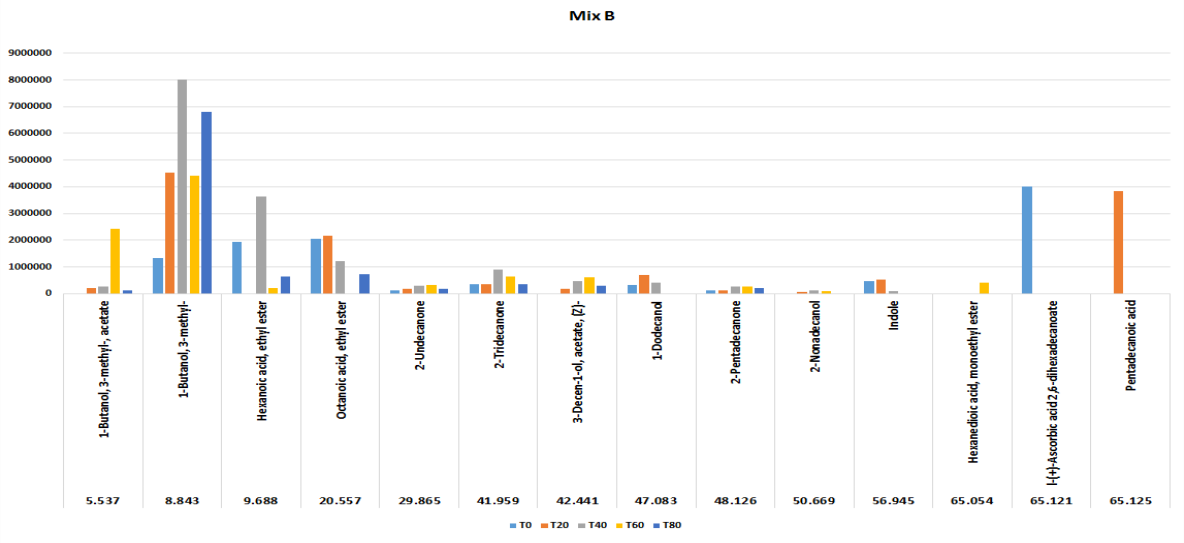

Fig. 2. Histogram of the GC-MS with SPME analysis for the Mix B during the 80 h.

4. Conclusions

The achieved results (Fig.1) strongly support the ability of the instrument to discriminate between the three different blends, follow the different stages of the culture development, and the change of the microbial composition of the blends. The obtained results with classical techniques (Fig. 2) have support the previous results showing and effective growth and development of the cultures.

Acknowledgements

The authors acknowledge the financial support from the European Commission the FP7 project “Sniffer for concealed people discovery (SNOOPY)” (grant agreement n. 313110).

References

[1] E. A. Grice, H. H. Kong, G. Renaud, A. C. Young, G G. Bouffard, R. W. Blakesley, T. G. Wolfsberg, M. L. Turner, J. A. Segre1, A diversity profile of the human skin microbiota, Genome Research 18 (2008)1043–1050.
[2] G. Sberveglieri, I. Concina, E. Comini, M. Falasconi, M. Ferroni, V. Sberveglieri, Synthesis and integration of tin oxide nanowires into an electronic nose, Vacuum 86 (2012) 532-535.
[3] V. Sberveglieri, E. Núñez Carmona,, A. Pulvirenti, Detection of microorganisms in water and different food matrixby by Electronic Nose, Sensing Technology: Current Status and Future Trends III, (2015) 243-258.
[4] M. Al-Omari, G. Liu, A. Mueller, A. Mock, R. N. Ghosh, K. Smith, T. Kaya, A portable optical human sweat sensor. J. Appl. Phys. 116 (2014) 183102.
[5] D. N. Fredricks, Microbial Ecology of Human Skin in Health and Disease, Journal of Investigative Dermatology Symposium Proceedings 6 (2001) 167–169.
[6] EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) European Food Safety Authority (EFSA), Parma, Italy, “Scientific Opinion on Dietary Reference Values for water”, EFSA Journal, 8 (2010) 1459.