Wound-State Monitoring for Burn Patients Using E-Nose/SPME System

Hyung-Gi Byun, Krishna C. Persaud, and Anna Maria Pisanelli

Array-based gas sensors now offer the potential of a robust analytical approach to odor measurement for medical use. We are developing a fast reliable method for detection of microbial infection by monitoring the headspace from the infected wound. In this paper, we present initial results obtained from wound-state monitoring for burn patients using an electronic nose incorporating an automated solid-phase microextraction (SPME) desorption system to enable the system to be used for clinical validation. SPME preconcentration is used for sampling of the headspace air and the response of the sensor module to variable concentrations of volatiles emitted from SPME fiber is evaluated. Gas chromatography-mass spectrometry studies prove that living bacteria, the typical infectious agents in clinical practice, can be distinguished from each other by means of a limited set of key volatile products. Principal component analysis results give the first indication that infected patients may be distinguished from uninfected patients. Microbial laboratory analysis using clinical samples verifies the performance of the system.

Keywords: Wound monitoring, electronic nose systems, SPME, clinical validation, bacterial infection.

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I. Introduction

Smell used to be a common diagnostic tool in medicine, and physicians were trained to use their sense of smell during medical training. However, odor diagnostics have been relegated to secondary status as a diagnostic method. Array-based gas sensor technology now offers the potential of a robust analytical approach to odor measurement for medical use. The technology has been used to examine odors emitted from the body, such as from breath, wounds, and body fluids, and to identify possible problems, such as bacterial vaginosis [1]-[3].

Wounds are injuries to body tissues caused by diseases, processes, or events such as burns, punctures, chronic leg or decubitus ulcers, or as the result of surgery. Wounds become infected when micro-organisms from the environment or from the patient's body enter the open wound and multiply. The symptoms related to an infection include abnormal flushing of the skin, heat, pain, tenderness, and abnormal odors, such as fruity odors that often indicate the presence of staphylococcus or foul odors due to the presence of gram negative bacteria. Standard techniques for microbiological detection are surface swabbing and wound biopsy culture. Surface swabbing is the most frequently used technique, mainly because it is quite inexpensive, but also because it is not invasive. However, swabbing can only assess surface infection, and it is time consuming. Biopsies are invasive and inconvenient.

There are three main processes by which wound infection may occur due to the presence of bacteria on a wound; namely, contamination, colonization, and infection [4], [5]. The earliest process is represented by contamination in which the pathogen introduces itself within the tissue. If the pathogen starts proliferating and multiplying, colonisation is taking place. It is

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reasonable to talk about infection when bacteria multiplication induces a host reaction. The proliferation and survival of micro-organisms depends on the efficiency of the host's immune system and the availability of the necessary chemical and physical factors. A concept developed especially for wounds is *critical colonisation*, which means a state in which micro-organisms are interfering with wound healing without inducing obvious clinical signs of infection.

Wound contaminations are likely to originate from the following three main sources [4], [5]:

- Environment-exogenous micro-organisms in the air or those introduced by traumatic injury,
- Surrounding skin-involving members of the normal skin micro flora, such as staphylococcus epidermidis, micrococci, skin diphtheroids and propionibacteria,
- Endogenous sources-involving mucous membranes, primarily the gastrointestinal, oropharyngeal, and genitourinary mucosae.

The normal microflora of the gut, oral cavity, and vagina are diverse and abundant, and these sources (particularly, oral and gastrointestinal mucosae) supply the vast majority of micro-organisms that colonize wounds. Detailed microbiological analyses of wounds demonstrate close correlation between the species found in the normal flora of the gut or oral cavity and micro-organisms present in wounds in close proximity to those sites. Microbiological analyses of colonized wounds of varied etiology demonstrate that anaerobes constitute 38% of the total number of microbial isolates. Many of the frequent wound colonizers, including Bacteroides, Prevotella, Porphyromonas, and Peptostreptococcus spp. survive for several days in the presence of air.

There are many different types of infection involving acute soft tissue, including surgical wounds, bite wounds, and burns. Infection is a major complication in burn wounds as it is estimated that up to 75% of deaths following burn injury are related to infection [5], [6].

Although exposed burned tissue is susceptible to contamination by micro-organisms from the gastrointestinal and upper respiratory tracts, many studies have reported the prevalence of aerobes such as P. aeruginosa, S. aureus, E. coli, Klebsiella spp., Enterococcus spp., and Candida spp [6], [7]. In other studies involving more stringent microbiological techniques, anaerobic bacteria have been shown to present from 11% to 31% of the total number of microbial isolates from burn wounds [7], [8]. Bacteroides spp. are found in the wounds of 82% of patients who develop septic shock, and such microorganisms may play a significant role in burn wound sepsis. Management of infection in burn wounds usually involves the use of topical and systemic antimicrobial agents, aggressive debridement of dead tissue, maximization of the immune response, and provision of adequate nutrition [9], [10].

The treatment of critically ill patients suffering from burns, chronic skin ulcers, or serious wounds is often complicated by infection. The current methods for detecting infection take two to three days before microbiological results are available to the clinician. It takes more time to determine which antibiotic or drug is appropriate. Consequently, patients suffer greater costs and extended hospitalisation.

Early detection of wound infection allows suitable antimicrobial interventions to be applied. Since infection always interrupts the normal healing process, efficient diagnosis and treatment of infection is required. Monitoring wound infection rates has also contributed to lowering the level of infection [10].

We are developing a fast reliable method for detection of microbial infection by monitoring the headspace from the infected wounds. In this study, we investigated bacterial volatiles from cultures of the most common strains of bacteria present in wounds, such as Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa in order to detect and identify potential volatile bio-markers of infection. Anaerobes were not considered in this initial study. In addition, we collected real samples (swabs or dressings) from patients to determine whether samples can be differentiated on the basis of the volatiles present.

The strategy is to use solid-phase microextraction (SPME) as a method of sampling and preconcentration of volatiles from the headspace. SPME is a technique to sample and preconcentrate the headspace using a fiber with a hydrophilic and/or hydrophobic coating [11]. The fiber adsorbs and/or absorbs the analytes in the headspace, and it is easy to handle.

The key marker volatiles were detected, and the volatile compounds emitted from wounds, where bacteria can often be found, verified the possibility of early recognition involved in infection using the SPME technique combined with gas chromatography-mass spectrometry (GC-MS).

The results obtained from this GC-MS study allowed us to build a mobile system for non-invasive wound monitoring using an array of gas and odor sensors, to be used for point of care monitoring of patients.

Sensors based on metal oxides were used, modified, and refined aiming to detect the most probable key markers for the bacteria types most frequently found in clinical conditions. For samplings from swabs or dressings from patients, an SPME approach was used for preconcentration of the low concentrations of volatile compounds emitted. An instrument was constructed that incorporated an automated SPME desorption system, sensor array, electronics, and data processing to enable the system to be used for clinical validation.

Samples of patients from the burns unit of the Wythenshawe Hospital in Manchester, UK, were analyzed in a preliminary study using an instrument based on gas and odor sensors. Primary results from the MOS sensor array give indications that infected patients may be discriminated from uninfected patients.

Microbial laboratory analysis provided support to validate the instrument for clinical application.

II. Experimental

1. GC-MS/SPME Experimentation

Many previous publications have reported on volatile organic compounds (VOCs) production by cultured bacteria. However, it is difficult to find reliable specific bacterial volatile markers in the literature [12].

In our case, we investigated bacterial volatiles from cultures and also swab and dressing samples taken from infected burns patients to determine whether there were detectable volatile compounds that could be used as markers.

Bacteria cultures were transferred into 40 ml vials containing the appropriate sterile medium, that is, brain heart agar (BHA) or tryptic soy agar (TSA), and incubated at 37°C for 24 h/48 h/72 h [13]. Also, swab and dressing samples were transferred into 20 ml vials containing BHA and TSA and incubated at 37°C for 1 h. Uninfected swabs were used as negative controls.

Qualitative analysis of the extracted volatiles was performed with a varian saturn GC-MS apparatus in the electron-ionisation (EI) mode.

The GC columns were Zebron ZB5 30 m×0.25 mm×1 μm and ZB Wax Plus 60 m × 0.32 mm × 0.5 μm (Phenomenex, USA). The GC oven program for the ZB5 column was as follows: held for 5 min at 50°C, ramped to 100°C at 25°C/min, held for 4 min, ramped to 150°C at 10°C/min, held for 6 min, then ramped to the finial temperature of 205°C at 5°C/min, and held for 10 min.

The GC oven program for ZB Wax Plus was as follwos: held for 1 min at 40°C, ramped to 200°C at 5°C/min, held for 5 min, then ramped to the finial temperature of 225°C at 8°C/min.

The inlet was operated in split less mode at 250°C, and SPME fibers remained in the inlet for 5 min. Bacterial fiber headspace preconcentration was carried out using SPME fibers polydimethlsiloxane/divinylbenzene (PDMS/DVB) 65 μm and carboxen/PDMS (CAR/PDMS) 75 μm (Supelco, Bellefonte, Pa, USA) for 30 min at 37°C.

Figure 1 shows the apparatus used for the analysis.

A search of Wiley 275, NIST, and Saturn mass spectral libraries allowed us to identify the volatiles produced by the bacteria that were then extracted and preconcentrated using the SPME fibers [14].

Fig. 1. GC-MS apparatus operated in the electron-ionisation mode used for analysis. Bacteria headspace sample analysis and SPME concentration was performed with Supelco fibers. Controls were carried out using agar media blanks.

2. E-Nose/SPME Experimentation

An electronic nose prototype system was developed comprising an automated SPME sampler and an array of sensors (see Fig. 2).

Sensors based on metal oxides were produced, modified, and refined with an aim to detect the most probable key markers for the bacteria types most frequently found in clinical conditions. The criteria for selection of the sensors was determined by the sensitivity and selectivity of the sensors to a limited number of the volatile compounds produced by living bacteria types that are defined as the most frequently found in clinical conditions during treatment of limited types of wounds.

The sensors in the system are commercially available metal oxide sensors fabricated by Figaro (TGS 2602, TGS 2610), Japan, and experimental SnO_2-based thin film sensors doped

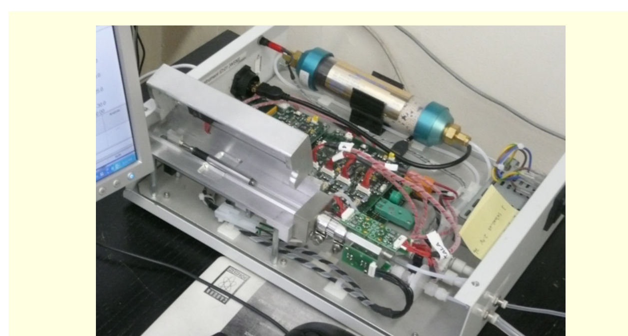

Fig. 2. Electronic nose prototype with combined sensor module, electronic air flow channel, and SPME fiber tray for real time wound stages monitoring. The system exposes the fiber to the heated sensor array desorbing the volatiles off the fiber directly onto the sensors to acquire data.

with Au, Cr, or WO developed by INFM-CNR, Italy and the Semiconductor Physics Institute, Lithuania [15], [16].

The instrumentation incorporated an automated SPME sampler, which allowed the SPME fibers to be contained and used for sampling via a plunger and locking system. The device acts as a mechanism for preconcentration of the sample, which effectively increases sensitivity of e-nose measurement.

Preconcentration of the headspace is achieved using SPME 75 µm CAR/PDMS fibers. The absorptive layer is identical to Supelco's specification with an adapted spring mechanism which allows for desorption within the instrument.

Swabs and dressing samples were transferred into 40 ml vials and kept at 4°C for e-nose/SPME analysis. SPME fibers CAR/PDMS were used to extract the headspaces, the extraction was performed at 37°C for 15 min. After sampling, the fiber was placed into an automated loader in the e-nose/SPME system that was controlled using an interfaced computer and specifically designed control software. The system exposed the fiber to the heated sensor array desorbing the volatiles off the fiber directly onto the sensors. The sensor system was linked to data processing software developed for real-time data acquisition of incoming data. The response of each individual sensor was measured over time after exposure to the volatiles.

3. Microbial Laboratory Analysis

Microbial laboratory analysis was carried out for swab and dressing samples taken from patients. The results from analysis can be used to verify the possibility of an e-nose/SPME system for detection of bacteria types at the early stage of wound infection.

III. Results and Discussion

The chromatograms from the GC-MS operated in an electron ionisation detector mole coupled with a specific headspace sampling technique known as SPME, allowing detailed analysis of potential biomarkers.

Figures 3 to 5 show interesting results. The three bacteria species gave different chromatograms, which is shown in Fig. 3. It was really difficult to distinguish between the strains of staphylococcus aureus and its subspecies methicillin-resistant staphylococcus aureus (MRSA), which were all grown on TSA, shown in Fig. 4. Figure 5 illustrates good reproducibility when analysis was replicated on the same bacteria cultures.

Considering the data from the bacteria headspace analysis, specific volatile markers were detected for the different bacteria species, but it was observed that alkane compounds

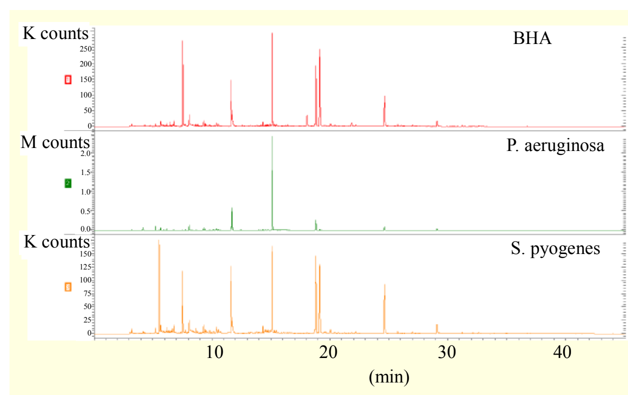

Fig. 3. Chromatograms of bacteria cultured for 24 h in BHA.

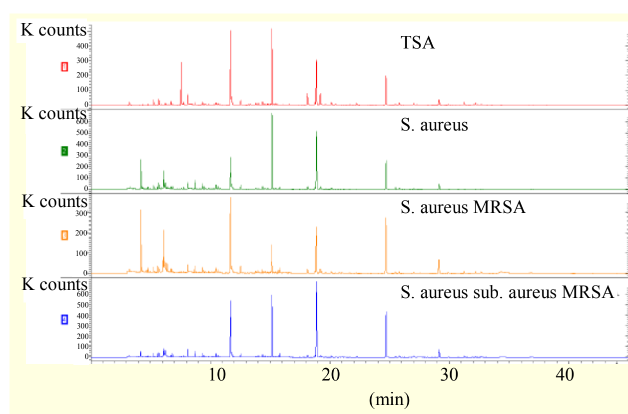

Fig. 4. Chromatograms of bacteria cultured for 24 h in TSA.

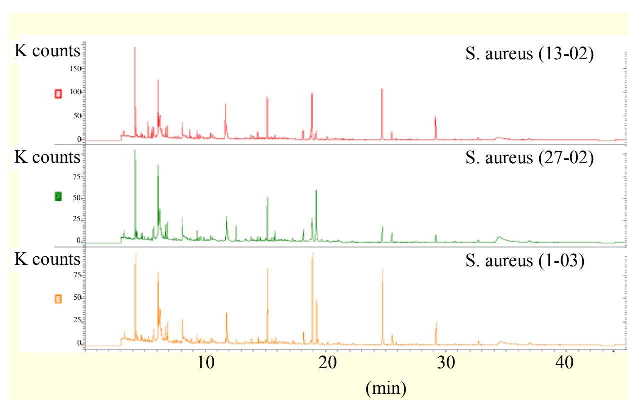

Fig. 5. Chromatograms of same bacterium analyzed on the three different dates.

(pentadecane, tetradecane, undecane) were not specific, as they were emitted by growth media (BHA or TSA). P. aeruginosa strains were found to emit ketones and alcohols. S. pyogenes emits ketones and alcohols that are different from P. aeroginosa. S. aureus strains produced ketones and fatty acids.

When analyzing the patients' sample chromatograms, other important considerations become evident. Judging from

Table 1. Microbial laboratory analysis results from samples taken from patients. X: no growth, P: Pseudomonas, S: S. aureus, St: Streptococcus.

Patients ID	Sample type	P	S	St	Comment
011	Swab	X	X	X	
	Dressing	X	500	X	Infected
013	Swab	X	X	X	
	Dressing	X	X	X	
014	Swab	X	X	X	
	Dressing	X	1,000	X	Infected
015	Swab	X	X	X	
	Dressing	X	X	X	
016	Swab	X	X	X	
	Dressing	X	X	X	
018	Swab	X	X	X	
	Dressing	X	X	X	
019	Swab	X	X	X	
	Dressing	X	X	X	
020	Swab	X	X	X	
	Dressing	X	X	X	
021	Swab	4,160,000	X	X	Infected
	Dressing	100,000	X	X	Infected
022	Swab	120,000	X	X	Infected
	Dressing	18,000	550,000	X	Infected

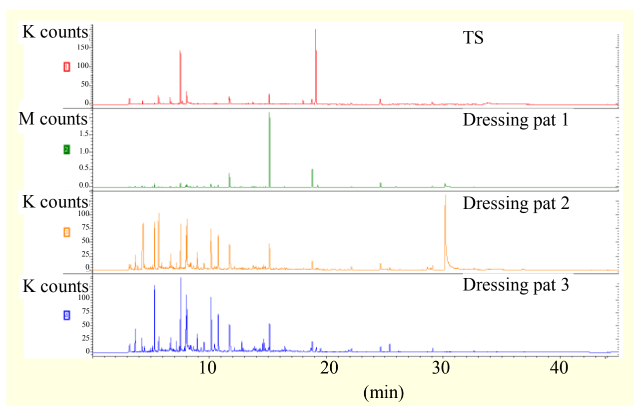

Fig. 6. Dressing chromatograms from patient samples.

chromatograms of patient swabs, there were no differences among the patients, although the first patient was infected according to the microbial laboratory analysis shown in the Table 1. This is due to a low intensity of volatile emissions (K counts) from swabs. For dressings, on the other hand, high signal counts were obtained. The chromatograms from the dressings from one patient who was infected show differences when compared to two uninfected patients as shown in Fig. 6.

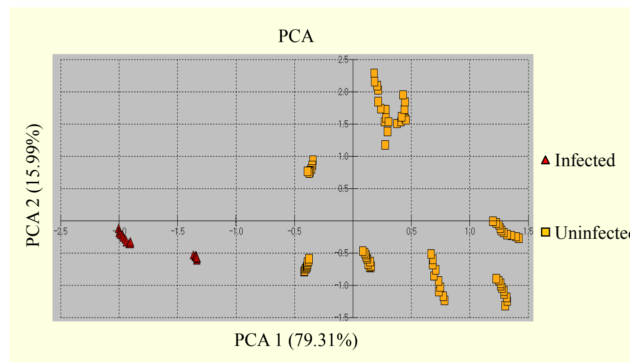

Fig. 7. PCA result of data obtained from the e-nose/SPME system from swab samples taken from patients.

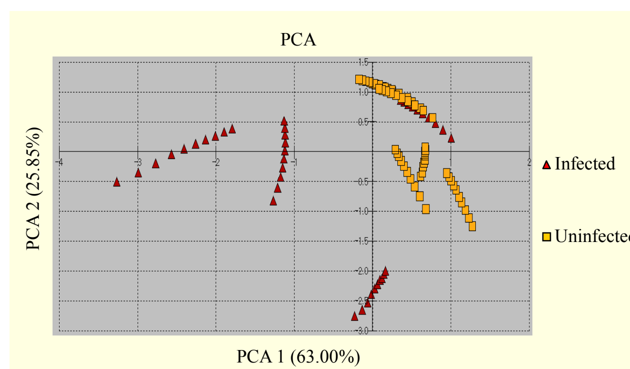

Fig. 8. PCA result of data obtained from the e-nose/SPME system from dressing samples taken from patients.

This point can be considered of great importance as it gives confidence about the valuable use of the dressing from the patients as a method to investigate possible infections.

The results obtained from chromatograms show that the collection of swabs and dressings enable detection of bacteria infecting wounds.

Microbial laboratory analysis was also carried out to identify bacteria infection from swab and dressing samples taken from patients. Table 1 shows the results of analysis, which can be used to verify the performance of the e-nose/SPME system capable of identification of infected patient's samples.

Figure 7 illustrates the principal component analysis (PCA) obtained from the electronic nose using the SPME headspace technique from swab samples taken from small samples of patients with serious burns. Two infected patients, diagnosed from microbial laboratory analysis, shown in Table 1, could be separated from others that were uninfected.

Analysis of dressing samples taken from patients was also done by the electronic nose using the same technique and is displayed in Fig. 8. This analysis clearly indicates that three patients were infected. This was not shown in swab samples. However, one patient was not included in the infected samples

class because low microbial counts were found in microbial laboratory analysis.

The PCA results of data obtained from the e-nose/SPME system give the first indication that infected patients may be discriminated from uninfected patients, and the microbial laboratory analysis for samples taken from patients verifies the performance of the system. The system is currently being validated on a large number of patient samples.

IV. Conclusion

Based on an array of electronic gas sensors, an odor recognition system was developed for detection of bacteria types at the early stage of wound infection. It was proved by GC-MS studies that living bacteria, typical infectious agents in clinical practice, can be distinguished from each other by means of a limited set of key volatile products.

To increase reliability of bacteria identification, SPME preconcentration was used for sampling the headspace air. Responses to variable concentrations of volatiles emitted from the SPME fiber were processed for evaluation of output parameters of the sensor module.

Discrimination between classes of volatile products was obtained by PCA analysis of the dynamic parameters of sensor responses to the headspace air of clinical samples collected by swabbing. Microbial laboratory analysis using clinical samples taken from patients demonstrated the acceptability of the system for discrimination between infected patients and uninfected patients.

Primary results at this stage appear to be promising. However, more clinical validation for the instrument is needed in hospitals where patients with wounds are treated.

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