Electronic Nose Based Bacteria Species Detection in Diabetic Foot Infection

by

Nurlisa Binti Yusuf @ Idris
(1331311015)

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School of Mechatronic Engineering
UNIVERSITI MALAYSIA PERLIS

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TABLE OF CONTENTS

LIST OF FIGURES

NO. **PAGE**

LIST OF TABLES

LIST OF ABBREVIATIONS

ANN	Artificial Neural Networks
ATCC	American Type Culture Collection
cfu	colony forming unit
DF	Discriminant Function
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectrometry
GDM	Gestational Diabetes Mellitus
H_a	The distribution is not normal
H_o	The distribution is normal
IDDM	Insulin-Dependent Diabetes Mellitus
KNN	K Nearest Neighbor
KS	Kolmogorov Smirnov
LDA	Linear Discriminant Analysis
LF	Lilliefors
LS	Least Square
MATLAB	Matrix Laboratory
MLP	Multilayer perceptron
MPN	Most probable number
MS	Mass Spectrometry
MSE	Mean Square Error
NaCl	Sodium Chloride
NIDDM	Non-Insulin Dependent Diabetes Mellitus

NIST	National Institute of Standards and Technology
PCA	Principal Component Analysis
PNN	Probabilistic Neural Network
RBF	Radial Basic Function
SMO	Sequential Minimal Optimization
SPME	Solid Phase Micro Extraction
SPSS	Statistical Package for the Social Sciences
spp.	Species (plural)
SVM	Support Vector Machine
SW	Shapiro Wilk
TIC	Total Ion Chromatogram
TOF	Time of flight
VOC	Volatile Organic Compound

Hidung Elektronik Berdasarkan Pengesanan Spesies Bakteria pada Jangkitan Kaki Pesakit Diabetes

ABSTRAK

Tesis ini membincangkan kajian asas pengesanan awal bakteria menggunakan hidung elektronik. Pengesanan awal jangkitan bakteria sangat perlu untuk memberikan rawatan yang berkesan untuk jangkitan kaki pesakit diabetes. Sehingga kini,kaedah klinikal berdasarkan kultur sampel adalah kaedah standard yang digunakan oleh ahli mikrobiologi untuk mengesan dan mengelaskan bakteria spesies. Pengkulturan sampel yang diambil daripada cebisan luka kaki pesakit diabetes boleh mengambil masa sehingga dua hingga tiga hari. Sebagai alternatif, hidung elektronik diperkenalkan untuk memberi diagnosis awal dan cepat kepada pesakit supaya rawatan yang sesuai dapat dijalankan. Projek penyelidikan ini menggunakan teknologi sensor sedia ada dalam bentuk hidung elektronik menggunakan kaedah pemprosesan data untuk mengenalpasti enam jenis spesies bakteria yang menjadi punca kepada jangkitan melalui bau yang terhasil. Kajian ini mempunyai tiga objektif utama iaitu untuk mengenal pasti spesies bakteria menggunakan medium pengkulturan yang berlainan, menyiasat keupayaan hidung elektronik untuk mengesan spesies bakteria kurang dalam medium "blood agar" dalam masa kurang dari 24 jam dan mengkaji secara *in-vitro* diagnosis spesies mikrob tunggal dan poli penyebab kepada jangkitan luka pada kaki pesakit diabetes. Hidung elektronik iaitu Cyranose320 mempunyai 32 susunan sensor gas digunakan dengan mengukur perubahan rintangan setiap sensor kimia yang boleh mengesan dan mengenalpasti bakteria mengikut sebatian organik meruap (VOC) yang terhasil. Bacaan Cyranose320 direkodkan dengan menghidu bau bakteria pada permukaan atas sampel yang dimasukkam dalam bekas khas yang telah ditutup rapi. Seterusnya, data yang dikumpul akan disimpan dalam fail data di dalam sistem komputer untuk diekstrak. Setelah data diekstrak, pelbagai eksperimen pengkelasan telah dijalankan. Perbandingan telah dibuat dan kesimpulan telah disediakan untuk melaksanakan pelbagai analisis data dan kaedah pengkelasan. Antara teknik pengkelasan digunakan di dalam kajian ini termasuklah "Support Vector Machine (SVM)", "K Nearest Neighbor (KNN)", "Linear Discriminant Analysis (LDA)" dan "Probability Neural Network (PNN)". 100% ketepatan dicapai menggunakan klasifier yang telah dipilih untuk mengenalpasti spesies bakteria yang dikultur di dalam tiga medium yang berlainan. Keputusan menunjukkan bahawa spesies bakteria yang berbeza dapat dikenalpasti oleh Cyranose320 walaupun menggunakan medium kultur yang berbeza untuk menghidupkan bakteria. Bagi pengesanan awal enam spesies bakteria, ketepatan terbaik ialah 96 %. Ini diperoleh menggunakan KNN dengan nilai k iaitu 2 dan 6 menggunakan jarak Euclidan dan Cityblock. Manakala untuk mengkaji secara *in-vitro* diagnosis spesies mikrob tunggal dan poli, ketepatan yang terbaik adalah di atas 90 % untuk kesemua klasifier yang digunakan. Oleh itu, kajian asas ini dapat dijadikan aplikasi dunia sebenar sekiranya teknologi ini berjaya dibangunkan. Kaedah dan teknik yang dibincangkan di sini adalah satu langkah ke arah matlamat untuk memperkenalkan sistem multi kelas sensor dalam kehidupan seharian. Kesimpulan tesis ini menunjukkan bahawa hidung elektronik berkebolehan mengesan dan mengelaskan spesies bakteria yang berbeza pada jangkitan luka kaki pesakit diabetes dengan keputusan yang meyakinkan yang boleh dibandingkan dengan prosedur standard yang sedia ada.

Electronic Nose Based Bacteria Species Detection in Diabetic Foot Infection

ABSTRACT

This thesis presents a fundamental study of early bacteria detection using electronic nose. There is a need for early detection of bacterial infection in order to give effective treatment for diabetic foot infection. To date, the clinical method based on sample culture is a standard practise used by microbiologist to detect and classify bacteria species. The cultured samples were taken from debridement of diabetic foot wound can take up to two to three days. Alternatively, identification of causative bacteria from their odours could provide an early and rapid diagnosis and therefore allow initiating appropriate treatment. This research project used an existing sensor technology in the form of an e-nose in conjunction with data processing and classification methods to classify six types of bacteria, common causal organism of diabetic foot infection from their odours. There were three main aims in this research study namely, to identify different bacteria species in different culture media using e-nose, investigate the ability of the e-nose to detect cultured bacteria species in blood agar medium in less than 24 hours and study *in-vitro* diagnosis of single and poly microbial species targeted for diabetic foot infection using e-nose. Cyranose 320 e-nose device which consist of 32 gas sensor array, measures the changes in resistance of each chemical sensor which can detect and classify bacteria according to their volatile organic compound (VOC). The sniffing process or e-nose measurements were performed immediately after placing the petri dish of bacteria suspension in a special stainless steel container. The odour data were collected and stored as numerical values within data files in the computer system. Once the dataset extracted, various classification experiments were performed. Comparisons were made and conclusions were drawn from the performance of various data analysis and classification methods. The classification methods used in this work include Support Vector Machine (SVM), K Nearest Neighbor (KNN), Linear Discriminant Analysis (LDA) and Probability Neural Network (PNN). 100% accuracy was achieved using all classifiers for identification of bacteria species in three different culture media. The results confirmed that possible to discriminate different bacterial groups on diabetic foot infection regardless of different culture media used for bacteria growth. For early detection of six bacterial species, the best accuracy was 96 %. This was achieved using KNN with k value of 2 and 6 using Euclidean and City block distance. For study in-vitro diagnosis of single and poly microbial species, the best accuracy was up to 90 % for all classifiers. Thus, this fundamental work on the classification of bacteria odours using e-nose can be a 'real world' application if this technology is successfully developed. The methods and techniques discussed here are one step towards the goal of introducing multi class sensor systems into everyday use. The conclusion of this thesis is that an e-nose can detect and classify different types of bacteria on diabetic foot infection with convincing results which are comparable to the existing standard procedure.

CHAPTER 1

INTRODUCTION

1.1 Background

According to the statistics in Malaysia, the diabetes disease is about 1.5 million in 2006 and this figure is predicted to increase to 2.3 million (Vithyatheri, Balakrishnan, & Loo, 2012); (Letchuman *et al.*, 2010) (Wild *et al*., 2004) due to life expectancy much longer and change in dietary habits. Therefore, government is estimated to spend about MYR 14.5 (USD 4.75) billion for helping 60,000 diabetes patients, each year. According to Ministry of Health, the healthcare for diabetes patients is far more expensive compared to one without diabetes (Vithyatheri *et al.*, 2012).

Normally, bacterial infection is the most common problem leading on to the diabetic foot complication and play main role in development of high risk gangrene and lower extremity amputation if not treated promptly (Zubair, Malik, & Ahmad, 2011). Diabetic foot infection is the foot of a diabetic patient that has the potential risk of pathologic consequences including infection, ulceration or destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral vascular disease and metabolic complications of diabetes in the lower limb (Zaini, 2000). There are three distinct stages of diabetic foot infections which were localized infection, spreading infection and severe infection (Edmonds, 2009). Usually, when signs of those clinical infections are present, osteomyelitis may take place which refers as bone infection caused by bacteria (Lipsky et al., 2012). Hence, early diagnosis of bacterial infections

and selection of appropriate antibiotics treatment based on its culture and antimicrobial susceptibility are very important. Immediate actions and appropriate antibiotic therapy can improve the treatment outcome of foot infection of the diabetic patient.

Currently, the conventional techniques used in the clinical laboratory to identify bacterial infection were ulcer swabs, curettage of the ulcer base, and needle aspiration after normal saline injection (Tascini et al., 2011). However, those techniques are often time consuming and may delay in getting diagnostic results (Fend et al., 2006). Another technique such as deep tissue biopsy immediately after surgery, is rather invasive and costly. Therefore, the use of an e-nose to identify a bacteria species is expected to provide the methods a faster diagnosis and non-invasive accurate result.

1.2 Problem Statement

To date, diagnostic system in Malaysia to diagnose bacteria species on diabetic foot infections take 2 to 3 days or more. The present techniques such as swabbed, aspiration, curettage, and tissue biopsy normally require a significant amount of time for culturing and identifying the causal pathogens before finalize the laboratory test results and pass to medical practitioner to start medications. Even when the most advanced microbiology was used, there is still a significant time lag between a culture specimen is taken and when the results are known to assist health care practitioners. Since many of these events occurs and keep on increasingly, therefore the result of diagnosing bacteria needs to get faster than usual (Ritaban Dutta, Das, Stocks, & Morgan, 2006).

Besides, selecting appropriate antibiotics for the treatment of diabetic foot infection is crucial. Identifying the optimal antibiotic choice requires careful consideration in terms of severity of infection, duration of wounds and previous antibiotic exposure. Previous issue on broad spectrum antibiotics has been discussed a

lot by previous researcher (refer Section 2.2.3). Basically, there were yet no data to suggest that a speeding the microbiologist diagnosis of diabetic foot infections by 2 to 3 days will improve patient outcomes. However, this e-nose study would improve patient care by improving or reduce drug resistance to infection and economical by using narrow spectrum antibiotics. More information on the patient's outcome can be study later with the availability of this technique or alternative method which is able to prescribe appropriate antibiotics at their first attempt.

Although there exists a number of studies investigating the use of e-nose in identifying the presence of bacteria in vitro, food safety, and clinical infections (refer Section 2.3, page 24-25), however this study is different with the other study because it involve with the poly microbial species rather than single bacterial species. Although this is preliminary experiment, hopefully this can contribute to further work to study the poly microbial infection. Besides that, the e-nose technique requires less than 24 hours to obtain the result.

Furthermore, this study involve with the multi-class technique were applied including recent classification approaches such as Support Vector Machine (SVM), k Nearest Neighbor (kNN), Linear Discriminant Analysis (LDA) as well as classical neural networks called Probability Neural Network (PNN). Thus, it is believe that this research is a novel since C320 can be as one of the options for rapid and accurate diagnosis of bacteria species detection of diabetic foot infection.

1.3 Research Objectives

The main objectives of this research are given as below:

a) To identify and classify different bacteria species in blood agar, Mueller Hinton and MacConkey media using Cyranose320.

b) To investigate the ability of the e-nose to detect cultured bacteria species in blood agar media as early as 24 hours.

c) To study the performance of e-nose in providing accurate classification for *in-vitro* diagnosis of single and poly microbial infection targeted for diabetic foot patients.

1.4 Research Scopes

The different bacteria species present on diabetic foot infections were diagnosed using an e-nose technology. The e-nose technology is designed for automated detection and classification of odours, volatile compounds and gases produced by bacteria. Thus, the scopes of this study are as follows:

1) Culturing bacteria in different media agar.

   For initial study, preparation and isolation of bacteria culture were carried out in the pathology laboratory at Hospital Tuanku Fauziah (HTF) from debridement of diabetic foot wound samples. Three different media agar which were blood agar, Mueller Hinton and MacConkey were chosed inthis study because media to detect three different types of bacteria species such as *E. coli*, *S. aureus* and *P. aeruginosa* which are usually a common cause of diabetic foot infection. Then, the samples were incubated for 24 hours in the incubator at 37 °C which is the optimum growth of bacteria.

2) Bacteria headspace analysis bacteria using e-nose.

   After 24 hour incubation time, the odour produced by the bacteria (headspace) was subjected to Cyranose320 for odour measurement. All the collected data from the Cyranose320 were analysed using various classifier algorithms. Later experiment the incubation time was reduced to less than 24 hours.

3) Statistical Analysis, Pattern Recognition and Artificial Intelligent using e-nose.

In order to obtain the accurate result, data pre-processing and dimension reduction technique such as LDA were performed prior to further analysis. Besides that, several Artificial Neural Networks (ANN) methods also applied such as SVM, PNN and KNN and compared to existing technique. At the end of the study, the result then were compared with standard culture methods and validated with Gas Chromatography Mass Spectrometry (GCMS).

1.5 Contributions of study

The first contribution in this study is to focus on study different culturing media to culture bacteria in bacteria plates. This study involves culturing wild-type bacteria and American Type Culture Collection (ATCC) standard bacteria strain in diabetic foot infection using e-nose. ATCC bacteria are a commercially available bacterium that is used as a standard reference in research. Although there were several reported findings (refer Section 2.3, page 23-24) on the ability of an e-nose to identify bacteria using the e-nose, however, it focused on other diseases rather than diabetic foot infection itself. Also, this study is attempting to investigate which method of neural network classifying the most suitable to be used in e-nose.

The second contribution in this study is to find early detection of bacteria species at 6 hours compared to current diagnostic techniques that would require at least 2 or 3 days to detect the bacteria species (Mazlina, Shamsul, & Jeffery, 2011). From the literature review (refer section 2.2.2.1, page 14-16) also stated that at least 24 hours of incubation time of bacteria. Therefore, this study takes at 6^{th} hour incubation time as the minimum observation of bacteria growth. Hopefully, this finding will be used by others to continue with repeated measurement.

The third contribution to this study investigates not limited on single bacterial species, but also on poly microbial infection due to multiple bacterial species represents in the real infection in diabetic feet (refer to Section 2.2.2.2). However, this is the first reported work study on mix bacteria species using Cyranose320 e-nose. The selection of bacteria used in this study is based on the established clinical data which is the predominant bacteria found on diabetic wound infections.

The final contribution of this study is developing the novel method for identifying bacteria species in diabetic foot infection. The techniques applied in this study (refer Section 3.3) is a non-invasive which is directly sniffing the sample in the sample container. It same goes as directly sniffing toward foot infection. If this method was successful may provide contactless on diabetic wound. This technique might bring one stage closer to the clinical measurement practice.

1.6 Chapter Outline

Chapter 1

This chapter covered the introduction, problem statement, objective of the study, brief explanation of the research scope and the contribution of this research.

Chapter 2

This chapter covered the literature review from the previous journal and articles, studies and the review about the diabetes mellitus disease and its complications, overview of foot infections including the clinical stage of infection and the microbiology of diabetic foot. Besides, the e-nose application, GCMS and other classification algorithm are also described in this chapter.

Chapter 3

The methodology and the design of the experiments are explained in this chapter.

Chapter 4

In this chapter, all findings of the experiments were identified based on the each research objectives mentioned in the Chapter 1. The discussion was begun with the GCMS analyses for validation of VOC released from bacteria. The purpose of this study is to confirm the results obtained from the e-nose analyses.

Secondly, the study involves the whole analysis, including the normality test, features extraction and classification evaluation in order to achieve the first and second objective of this study, which is to identify different bacteria species in three different media culture and early detection and classification causative bacteria on foot infected.

Finally, the rest of the analyses were focusing on study the performance of e-nose in providing accurate classification for *in-vitro* diagnosis of single and poly microbial infection were discussed in detailed.

Chapter 5

The conclusion of the experiment will be covered in this chapter together with the future works to enhance this research in related medical field.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus Disease

Diabetes mellitus is the lifetime disease which contributes the highest cause of death in some countries in the world (Beaglehole & Han, 2004; P. Wang, Tan, Xie, & Shen, 1997). Usually, it occurs when the pancreas (small organ that sits behind the stomach) produces very little insulin or does not efficiently produce insulin into the body (Al-Qazaz et. al., 2011). Insulin is a hormone made by the pancreas that allows body to turn blood glucose into a kind of energy to the cells. If the insulin hormone cannot be produced by the body, the sugar cannot pass from the blood into the cells to form energy (Pedersen & Cobelli, 2014). The classification of diabetes mellitus is summarized in Table 2.1.

Table 2.1: Classification of diabetes mellitus disease (adapted from Robert G. Frykberg, Thomas Zgonis, 2006)

Type of diabetes	Classification of diabetes
I	The pancreas produced little or no insulin (insulin deficiency).
II	The pancreas still produced insulin, but the body develops resistance to insulin.
Gestational	High blood glucose level during pregnancy

Type I diabetes known as insulin-dependent diabetes mellitus (IDDM) is an autoimmune process in which the body's immune system itself attacks and destroys the insulin in the pancreas from producing cells. When sugar cannot pass into the cells, it just keeps circulating and building in the blood and the body's cells literally starve to

death (Bader, 2008). Usually, this disease occurs in children and young adults (Woo, 2008). Patients need injections of insulin every day in order to control the levels of glucose in their blood. They will die if do not have access to insulin (Siewko et al., 2013).

Non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes is classified as Type II diabetes disease. It is accounted for 90% to 95% of all cases diagnosed (Robert G. Frykberg, Thomas Zgonis, 2006). Type 2 diabetes is characterized by hyperglycaemia in the presence of high blood sugar builds up in the blood due to peripheral insulin resistance. Usually, this type of disease occurs after the age of 40. It may occur at earlier age especially in populations with high diabetes prevalence. It may remain invisible for many years and sometimes this type of disease can be diagnosed incidentally through urine glucose test and systolic and diastolic blood pressure (Abougalambou & Abougalambou, 2013). Besides, obesity is one of the contributing factor of this type of disease which itself can cause insulin resistance and lead to elevated blood glucose levels (Woo, 2008).

Moreover, another type of diabetes which is known as gestational diabetes mellitus (GDM), recognized by a high blood glucose levels during pregnancy. It affects 2-12% of all pregnancies (Page, Romero, Buchanan, & Xiang, 2014) and carry a lifetime risk of the foetus (Li et al., 2014). Women with GDM are at increased risk of metabolic diseases together with obesity, high blood pressure, dyslipidaemia, insulin-resistance, cardiovascular disease and Type II diabetes. Furthermore, women with GDM has 7 times the risk of getting Type II diabetes future in life than women without GDM (Much, Beyerlein, Roßbauer, Hummel, & Ziegler, 2014).