

# Biosensors as Point -of-Care-Testing (POCT) for Diagnosis and Monitoring of Periodontitis: A Comparative Review

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**Abstract. Introduction:** Periodontitis is a non-reversible inflammation of the peridontium, that if left untreated will eventually lead to teeth loss. Currently, the routine diagnosis of periodontal disease is primarily based on clinical (oral inspection, palpation and probing) and radiographic measures of periodontal tissue loss. Whilst these parameters are very useful, they only provide information on tissues destruction that already taken place hence are of limited use for prevention and early diagnosis. More non-invasive diagnosis methods have been developed, utilizing saliva from patients and immobilization of specific analytes onto a biosensor platform.

**Objective:** This study aimed to compare various types of biosensors as point-of-care- testing for diagnosis and monitoring of periodontal disease. **Methodology:** A systematic literature search was performed in three public databases; Pubmed, Google Scholar and Science Direct. Search was carried out without any restrictions on date of publication however restricted to original research that developed biosensor platform for detection of periodontal disease using patient saliva samples. **Results:** Literature search resulted in seven studies that described various biosensors as point-of-care device capable of detecting specific analytes or biomarkers that is associated with periodontitis. Two of these devices used proteins derived from bacteria which causes periodontitis. Each biosensor has different detection mechanisms and varies in detection limit of analytes tested. **Conclusion:** Development of biosensors capable for detection of saliva-based analytes as a non-invasive diagnosis point-of-care test could serve as an adjunct to complement existing routine diagnosis method.

**Keyword:** periodontitis, saliva, biosensor, diagnosis, point-of-care testing

## Introduction

Globally, severe periodontitis has been reported affecting up to 15% of the populations (Petersen & Ogawa, 2000) making it the sixth most prevalent disease in the world, ranked higher than chronic diseases such as cardiovascular disease. Increasing trends of severe periodontitis is also observed in Malaysia (Mohd Dom *et al.*, 2013). The decennial survey carried by Ministry of Health Malaysia (MOH) in 2010 shown an increased in disease prevalence of moderate and severe periodontitis to 30.3% and 18.2% respectively. Subsequently, 9 in 10 Malaysian adults have experienced periodontal disease and dental caries according to the latest MOH statistics in 2016. This increasing trend of periodontal disease prevalence reflects the dire need of early diagnosis especially targeted towards more susceptible groups such as the elderly population who are at higher risk. The diagnosis of periodontal disease or also known as periodontitis relies heavily on the clinical parameters such as probing depth, attachment level, bleeding on probing (BOP) and radiographic assessment of alveolar bone loss, in some cases might be supplemented with microbial analysis. These methods may not be precise enough to differentiate between general chronic periodontitis and aggressive periodontitis, and only allowing retrospective diagnosis of attachment loss. Conventional diagnosis methods of periodontitis are based

on the measurement of clinical attachment loss and radiographic evaluation of alveolar bone loss (Amit De et al., 2018).

The current routine clinical methods have been widely used because it is easy to handle, safe and established. But, these methods are time-consuming, can only be suited with clinical set-ups and produce minimal details since they only detect periodontal changes that have happened instead of current disease progression (Ji et al., 2015). Due to these disadvantages, there is a need to explore novel methods for a more predictive investigation (probing) in addition to reduce subjective clinical interpretations. For radiographic evaluation, a new 3D imaging tool known as cone beam computer tomograph for examination of alveolar with significantly lower levels of radiation has been developed. This new method is more reliable whereby the findings can be used in the identification and classification of bone defects compared to intraoral and panoramic radiography (Zhang et al., 2017).

### **Application of saliva in the diagnosis of periodontitis.**

Recently, saliva has been studied for diagnosis of periodontitis due to its feasibility, non-invasive mode of sampling and ease of frequent collection with minimal discomfort to the patient. Saliva contains promising biomarkers that have been shown to correlate with periodontal clinical parameters (AlMoharib et al., 2014; Miller et al., 2010; Taylor, 2014). However, application of saliva alone for diagnosis may possess some limitation, for example, inability to detect disease activity at each individual tooth site. For this, complete diagnosis of periodontitis needs to be supported by existing routine methods. Another approach which is gaining more attention nowadays is combination of saliva sampling with further applications onto point-of-care testing (POCT) platform as an adjunct to diagnose periodontitis. Generally, the POCT platform provides the ability to be used outside clinical setting, such as in remote areas in contrast to routine methods that is confined to the clinics (Ji et al., 2015; Song et al., 2014). Realising the future prospect of combinatorial saliva and POCT platform for periodontal diagnosis, this minireview was performed to compile all existing and developed POCT biosensors used for this purpose.

### **Biomarkers of Periodontitis**

The used of biomarkers for detection of periodontitis not only determine sensitivity and specificity of targeted analytes associated with periodontal disease progression, but also predict the progression of periodontitis (Gul et al., 2016). An ideal biomarker must able to diagnose the presence of periodontitis, shows the severity, able to monitor the response of the disease to treatment and able to predict the progression of the disease. In general, the saliva-based biomarkers for periodontitis are divided into two categories; which are bacteria-derived and host-derived salivary biomarkers (Ji et al., 2015).

#### **Bacteria-Derived Salivary Biomarkers**

There are several studies associating presence of specific bacteria with periodontitis such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Tannerella forsythia*. These studies used 16S rRNA gene as specific targeting for detecting presence of bacteria on periodontal affected sites (Griffen et al., 2012; Göhler et al., 2014). Some study also uses gingipains, one of the specific proteases secreted by *P. gingivalis*. as a detection biomarker. Other proteases that are secreted by this bacteria species are peptidyl arginine deiminase, hemagglutinin and fimbriae. These virulence bacterial proteins promote the existence of *P. gingivalis* found within periodontal pocket that leads to degradation of the teeth supporting tissues (Alhogail et al., 2018). Another study has reported detection of antimicrobial peptides (magainin I) as biological recognition elements to detect the presence of bacteria (Hoyos-Nogués et al., 2016).

#### **Host-Derived Salivary Biomarkers**

The periodontitis progression results in soft tissue destruction which further release several enzymes and proteins involved in tissue degradation into the saliva (Ji et al., 2015). There are several cytokines

that are released during this process, facilitated by neutrophils and macrophage to kill and lyse invading bacteria, for example interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), human neutrophil elastase, cathepsin-G, odontogenic ameloblast-associated protein including proteases such as MMP-8, MMP-9, salivary peroxidase and prostaglandins. Timely detections of these host-factors enable diagnose of periodontitis, predict disease progression and patients' response to treatment (Ivnitski et al., 2003; Lee et al., 2019; Mohseni et al, 2016; Taylor et al., 2019; Wignarajah et al., 2015). Table 1 shows salivary biomarkers and types of sample collection from original research articles that have been included in this minireview.

**Table 1. Saliva-based biomarkers used in POCT diagnosis of periodontal disease.**

<b>Biomarkers</b>	<b>Types of sample</b>	<b>References</b>
MMP-8	Saliva	Taylor et al., 2019
MMP-9	Saliva	Mohseni et al., 2016
Human neutrophil elastase and cathepsin-G	Commercial saliva and gingival crevicular fluid (GCF)	Wignarajah et al., 2015
Odontogenic ameloblast-associated protein	Saliva	Lee et al., 2019
Salivary peroxidase	Saliva	Ivnitski et al., 2013
Antimicrobial peptides (magainin I)	Artificial saliva	Hoyos-Nogués et al., 2016
Gingipains protease	Saliva and GCF	Alhogail et al., 2018

### **Point-of-care-testing Platforms and Their Mechanisms**

Point-of-care-testing (POCT) platforms for periodontitis diagnosis have been established to better represent the current state of periodontal destruction, assess the risk of disease development and evaluate the recovery of periodontitis. The advantages of this platform compared to routine clinical methods are low cost, rapid detection, easier application and not restricted to clinical settings (Hu et al., 2014). POCT are divided into two categories which are lab-on-chip (LOC) and paper-based platforms.

#### **Lab-on-chip (LOC) Platforms**

Currently, LOC platforms for periodontitis diagnosis are mostly focused on immunoassays for detection of specific biomarkers.

#### **Impedimetric antimicrobial peptide-based sensor**

This biosensor detected presence of bacteria based on specific peptide. The biosensor combined the use of miniaturised and integrated impedimetric transducers and AMPs to monitor bacterial colonisation with high sensitivity. The frequency range that has been used was 100 Hz – 1000 kHz with 100 mV (amplitude) voltage excitation using Quadtech 7600 Plus LCRMeter. The biosensor capable to detect the presence of bacteria at 10<sup>1</sup> colony forming units (CFU)·mL<sup>-1</sup> in KCl solution and 10<sup>2</sup> CFU·mL<sup>-1</sup> in artificial saliva. The AMPs coupled with 3D-IDEA biosensors has shown rapid implementation, label-free, and sensitive enough to detect periodontitis-associated pathogenic strain, *S. sanguinis*. The AMP-based sensor array able to detect a broad spectrum of bacteria and acts as tool to prevent the initial formation of biofilm and coordinated related implant-infection commonly found in periodontitis patients (Hoyos-Nogués et al., 2016).

#### **Surface Plasmon Resonance (SPR)**

SPR is used to monitor antigen-antibody interaction by means of detecting changes of refractive index. The sensor will detect changes once analyte molecules and specific immobilized ligand is bound on sensor chip. In this application, the relationship of matrix metalloprotein-9 (MMP-9) which is an example of antigen and anti-MMP-9, acting as corresponding antibody were determined by equilibrium constant ( $K_D$ ) (0.4 nM), maximum binding capacity ( $R_{max}$ ) (680  $\mu$ RIU) and dock binding free energy

( $\Delta G_b$ ) (- 53.51 KJ/mol. This application reported MMP-9 concentration within the range of 10 – 200 ng/mL and the detection limit of 8 pg/mL. The sensor used metal film with ~ 50 nm thickness which is run with buffer containing 7.8 mM  $\text{NaH}_2\text{PO}_4$ , 8 mM  $\text{Na}_2\text{HPO}_4$ , 137 mM NaCl, 0.1 mM  $\text{CaCl}_2$ , 3mM KCl, 1.5 mM  $\text{KH}_2\text{PO}_4$  and 0.02 % (v/v) Tween 20, pH 7.8. This approach has shown that the SPR immunosensor as a label-free sensing system is a practical procedure to measure and monitor MMP-9 in biological samples. In addition, this method able to provide better understanding in the interaction of molecules in life sciences, biotechnology and medical sciences (Mohseni et al., 2016).

### **Amperometric Biosensor**

This amperometric biosensor is coated with salivary peroxidase, serving as the detector and assembled with a micropipette for injecting specific quantity of liquid sample into flow detector connected to an electronic book. The electronic book is composed of an amplifier, a special design peak detector, microprocessor and display. The operations of this biosensor involved sample injection, electrochemical detection and data processing. It is used for single action only via pressing the plunger of the micropipette. Only minimum volume of samples and reagents are needed due to elimination of the peristaltic pump. The lowest detection limit of peroxidase of this biosensor was 0.5 ng/ml and the response time of the sensor was 2 -3s. Application of this amperometric biosensor, coupled with commercial available kits and equipped with peroxidase-labeled conjugate enable new portable electrochemical biodetectors for enzyme immunoassay such as hormones, drugs, viruses, antibodies and bacteria (Ivnitski et al., 2003).

### **Surface Acoustic Wave Technology (SAW)**

A SAW biochip is coated with specific antibodies to measure MMP-8 concentration in periodontal disease patients. The SAW is capable to detect analyte in control box using analogue signal. The software is installed in the laptop/ PC to process the conversion of signal from the control box. The biochip is composed of input and output gold electrode linked by a gold film coated sensing area that built on a plane piezoelectric quartz crystal. This biosensor setup able to give a shear horizontal SAW excitation to a defined wavelength and signal frequency. In addition, this biosensor assay is able to produce good quality performance as compare with ELISA assays for MMP-8. The detection limit that produced by this biosensor was 62.5 ng/mL with the effectiveness range of 0 – 1000 ng MMP-8/mL. The advantages of the biosensor is real-time measurement, ability to modify protocols for chip preparation and reduction in time for analysis washing steps within 15 minutes (Taylor et al., 2019).

### **Paper-based Platforms**

Paper-based platform is one of the cheapest technology with the simple fabrication and is independent from external instruments.

### **Magnetic Nanoparticle Biosensor**

The use of magnetic nanoparticle as diagnostic biosensor is made possible by coupling the application with two types of salivary biomarkers such are human neutrophil elastase and cathepsin-G. This biosensor is able to measure the proteolytic activity using magnetic nanoparticles and specific probes. The probes are coated with specific proteases substrates which are bound to a magnetic bead from one end to the gold sensor surface by the other end. When intact, the colour of sensor will change from gold to black. During proteolysis, the magnetic beads will cleave once they are attracted to an external magnet which reveal the golden colour sensor surface that can be observe from naked eye. This method able to reach sensitivity as low as 100  $\text{fg}\cdot\text{ml}^{-1}$  for cathepsin-G and 1  $\text{pg}\cdot\text{ml}^{-1}$  for human neutrophil elastase. This approach has introduced the useful of biochip array as low-cost POC devices (Wignarajah et al., 2015).

### **Sandwich-type Lateral Flow Strip (LFS) Biosensor**

The sandwich-type biosensor allowed a cognate pair of aptamers binding with human odontogenic ameloblast-associated protein (ODAM). The immunoassay technology that LFS used is nitrocellulose membrane, coloured nanoparticles and specific antibodies. The sensor will operate once the sample is added, followed by sample flowing into the test device and passes through the conjugate pad into the nitrocellulose membrane and finally onto absorbent pad. This study indicated the possibilities for a cognate pair of aptamers on POC biosensors that ensures easy, fast and non-invasive saliva-based diagnosis of periodontitis that can resolve existing diagnostic methods and enhance the health care system. The biosensor showed detection limit in buffer (8.32 nM) and saliva samples (14.59 nM). One of the advantage of this biosensor, is the ability to detect faster readout in human saliva samples (Lee et al., 2019).

### **Magnetic-nanobead-based Assay**

Magnetic-nanobead-based assay labelled with gingipain-specific peptide is used to diagnose periodontitis -related infections caused by *P. gingivalis*, one of the most common pathogenic bacteria. The gingipains is the specific proteases that is released by the bacteria during inflammatory stages. The sensor is coupled with gingipain biomarkers which were immobilized on a gold biosensing platform via gold-thiol linkage which resulted in the color of gold layer changed to black. When the immobilized substrates being cleaved by gingipain, the magnet will be attracted to the magnetic-nanobeads-peptide fragments resulting in observed golden surface color. This biosensor has rapid action and higher sensitivity and specificity, able to detect as low as 49 CFU·mL<sup>-1</sup> of *P. gingivalis* within 30 s (Alhogail et al., 2018).

### **Conclusion**

Seven newly developed biosensors that were used to diagnose periodontitis-related biomarkers have been discussed in this minireview. These sensors are specifically suitable for salivary based analytes, thus enabling less invasive mode of diagnosis. Although they are still in the early development stages, many of them shows the potential and capability to be used as point of care testing devices, enabling diagnosis of periodontal disease to be done outside clinical settings. Choosing a suitable biosensor and biomarkers is critical depending on the criteria of periodontal monitoring required.

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