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Review article:

Point of care – A novel approach for fast periodontal diagnosis

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Abstract

Periodontal disease, a known silent disease which seldom causes pain only when the disease has progressed in which advanced treatment approach is a must. To safeguard from periodontal disease there is a need for practical supplementary tests to diagnose it in the dental office. It includes intraoral observation of gingival inflammation, periodontal probing depth, loss of clinical attachment, radiographic bone loss, and signs and symptoms of active disease including ulceration and suppuration. Diagnosis of a disease and selecting an appropriate treatment should ideally rely on the knowledge that disease is in fact active and progressing. A common dilemma faced by dental practitioners is the differentiation between active periodontitis and quiescent disease that is superimposed on an already reduced periodontium.

Introduction

To safeguard from periodontal disease there is a need for practical supplementary tests to diagnose it in the dental office. Although, periodontal disease is largely a host-innate immune response to bacterial infection its diagnosis in the average private dental practice is currently limited to traditional assessment. This evaluation includes intraoral observation of gingival inflammation, periodontal probing depth, loss of clinical attachment, radiographic bone loss, and signs and symptoms of active disease including ulceration and suppuration.[1] Currently, determining the severity of chronic periodontitis, as classified by the World workshop of Periodontology 2017 [2] is based on loss of clinical attachment as measured by a periodontal probe and radiographic bone loss. However, both these methods are unable to diagnose future periodontal disease progression.

Diagnosis of a disease and selecting an appropriate treatment should ideally rely on the knowledge that disease is in fact active and progressing. A common dilemma faced by dental practitioners is the differentiation between active periodontitis and quiescent disease that is superimposed on an already reduced periodontium.

Need for newer methods

The principles of these new diagnostic tests largely rely on the detection of the markers of disease activity. The term "markers of disease" basically consists of three separate categories; (1) indicators of current disease

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activity; (2) predictors of future disease progression; (3) predictors of initiation of future disease at a currently healthy site.[3]

Biomarkers are quantifiable and measurable biologic parameters that serve as indicator for health and physiology-related assessments[4]. The biomarkers provide "signature" of the health state; they are found in the biological fluids such as blood, urine and more recently saliva [5]. It is rightly said that oral fluid is the mirror of periodontal health and it serves as medium to provide clinically relevant information since it contains biomarkers specific for periodontal diseases. Some of the oral fluid biomarkers like proteins of host origin (e.g., enzymes and immunoglobulin's), host cells (e.g., PMNs), bacteria as well as bacterial products, ions, hormones and volatile compounds have been studied for periodontal diagnosis [6].

Traditional methods of periodontal diagnosis (figure 2)

- Periodontal probing
- Radiographic examination

Sources used for sample collection

Saliva

Saliva is a biofluid which is readily accessible and can be collected by totally non-invasive method. Saliva offers many advantages as it is readily available, contains a rich array of diagnostic biomarker molecule, non-invasive method of sampling and ability to obtain rapid and reliable results. Saliva has also proved to be beneficial as compared to blood because it is easy to handle saliva as it does not clot and also chances of accidental transmission of infectious disease during its collection is less than blood samples[7]. However, one of the major limitations of using saliva is that as compared to saliva and serum the informative analytes generally are present in lower amount therefore, assays need to be highly sensitive[8]. The origin of saliva determines its composition and is influenced by various environmental and psychological stimuli. Thus, qualitative analysis of saliva markers can be reliably achieved but to quantify these markers is the real problem. Apart from these, presence of mucins and cell debris makes saliva a challenging fluid to work with.

Biomarkers in saliva		
Markers of periodontal inflammation	**Markers of alveolar bone loss**	**Collagen breakdown products**
Prostaglandin E2	Alkaline phosphatase	Aspartate aminotransferase
β - glucuronidase	Osteoprotegerin	Alanine aminotransferase
IL- 1β	Osteocalcin	TIMPs
IL- 6	Collagen telopeptidase	MMPs
Tumor necrosis factor- α	Pyridinoline cross- links of type 1 collagen	α2- macroglobulin
Matrix Metalloproteinase (MMP-8,9,13)	RANKL	
	Osteonectin	

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Gingival crevicular fluid

GCF can be frequently used for biomarkers as it easily obtained from the oral cavity. Chapple I stated the advantages of using GCF: "The biomarkers found in GCF indicate the presence or absence of periodontal pathogens, gingival and periodontal inflammation, the host inflammatory-immune response to specific pathogenic species and host tissue destruction". The disadvantages of using GCF are that it requires multiple samples of individual tooth sites and extensive laboratory processing, thereby making it expensive and time consuming[9].

Although, GCF has several diagnostic advantages because of the appearance of inflammatory mediators and tissue-destructive molecules in it, the procedure of collection and analysis makes it difficult to be used as a chairside diagnostic medium [Table/Fig-4]. GCF collection is laborious and technically demanding requiring special equipment for calibrating and measuring fluid volumes. There is also a possibility of GCF being contaminated with blood, saliva, or plaque[10].

Biomarkers in gingival crevicular fluid

Inflammatory and immune products	Bacterial proteases	Host derived enzymes	Tissue breakdown products	Bone specific proteins
Prostaglandin E2	Alkaline phosphatase	Alkaline phosphatase	Glycosaminoglycan	Pyridinium crosslink urine pyridinoline
Cytokines	Amino peptidases	β - Glucuronidase	Hyaluronic acid	Pyridinium crosslink collagen peptide
Antibacterial antibodies	Chondroitin sulphatase	Elastase	Chondroitin- 4-sulfate	Tartrate resistant acid phosphatase
Acute phase proteins	Collagenase	Cathepsins	Chondroitin- 6-sulfate	Hydroxyproline
Complement	Fibrinolysin	Serine protease	Dermatan sulfate	Galactosyl hydroxylysine
Vasoactive intestinal	Glucosidases	Nonspecific neutral	Hydroxyproline	Glycosaminoglycans
Peptide		Proteinases		
Neurokinin a	Haemolysin	MMPs 3,8,13	Fibronectin fragments	Osteonectin and bone phosphoprotein
Neopterin	Hyaluronidase	Aspartate aminotransferase	Connective tissue and bone proteins	Osteocalcin
Platelet activating factor	Phospholipase	Myeloperoxidases	Type 1 collagen peptides	
	Hydroxyproline	Lactate dehydrogenase	Polypeptide growth factor	

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Point of care diagnostics

International standard ISO 22870, *Point-of-care testing (POCT) - Requirements for quality and competence*, defines POCT as: "testing that is performed near or at the site of a patient with the result leading to possible change in the care of the patient".

High specificity and sensitivity are essential requirements of a good diagnostic marker which could be used chairside or in a home use device. The widespread use, simplicity, level of reliability and relative low cost of a home-used pregnancy test is the path to follow in periodontics[11].

Clinical Chairside Diagnostic Platforms

Classification	Platforms	Sample	Targets	Principle	Merits	Demerits
Microbiological	Omnigene	Laboratory and clinical specimens	Aa, Pg	DNA hybridization	Quantification	(i) Dependence on fully licensed lab (ii) Time-consuming
	Evalusite	Subgingival samples	Aa, Pg, Pi	Sandwich enzyme immunoassay	Visual detection and differentiation of antigens	(i) Multistep operation (ii) Subjective readout (iii) low sensitivity in shallow pockets
	PerioScan	Subgingival plaque	Pg, Td	BANA reaction	Detection of volatile sulphur-containing compounds in halitosis patients	(i) Quantification is not possible (ii) unable to differentiate between bacterial species
	Perio 2000	Subgingival samples	Pg, Pi and Tf	VSCs	Easy to use	Quantification is not possible
Biochemical	Periocheck	GCF	Neutral proteases	Enzymatic digestion reaction	Visual readout of levels of neutral proteases	(i) Quantification is not possible (ii) low specificity for PMN

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						collagenase
	PerioGard	GCF	AST	Enzymatic catalysis reaction	Visual readout of AST activity, fast	(i) Poor differentiation between colours (ii) complex procedure (iii) Poor discrimination of attachment loss
	PocketWatch					Susceptible to extracellular matrix effects
	DentoAnalyzer	Saliva GCF	Various related bacterial	Sandwich enzyme immunoassay	Quantification, fast	Large size
	Prognostik	GCF	Elastase	Immunofluorescence	Can identify active disease sites	Further clinical trials required
Aa- actinomycetemcomitans; Pg- porphyromonas gingivalis; Td- treponema denticola; Pi- prevotella intermedia AST, aspartate aminotransferase; BANA, N-benzoyl-DL-arginine-2 naphthylamide; PMN, polymorphonuclear leukocyte						

What is new?

'Lab-on-a-chip' and microfluidic devices have emerged as a great hope in managing oral fluids such as saliva and gingival crevicular fluid and they also determine patient's periodontal disease-risk profile, current disease activity and response to therapeutic interventions. This approach in turn proves to be useful in a chronic infectious disease such as periodontitis in terms of monitoring of episodic nature of this disease and in making clinical decision[7].

Other point of care diagnostics

a. Oral fluid nanosensor test: A new POC device to detect oral cancer in saliva was developed by the University of California, Los Angeles (UCLA) Collaborative Oral Fluid Diagnostic Research Laboratory, led by Dr. David Wong[12,13]. This is an automated POC device that is designed for the electrochemical detection of multiple salivary proteins and nucleic acids. It is an ultra-sensitive and ultraspecific micro electromechanical system which simultaneously and precisely detects these proteins and nucleic acid. The product is Oral Fluid Nano Sensor Test (OFNASET). Four salivary mRNA biomarkers (SAT, ODZ, IL-8 and IL-1b) and two salivary proteomic biomarkers (thioredoxin and IL-8) in saliva are detected in this system[7]. The OFNASET is actually a screening device for detecting oral cancer[13]

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b. Electronic taste chips: Researchers at Rice University in Houston, Texas, are developing a lab-on-a-chip system, which will differentiate between healthy and periodontally diseased individuals based on the CRP levels[7]. This microchip based detection system is used for measuring analytes (acids, bases, electrolytes and proteins) in solution phase. This novel system is called an Electronic Taste Chip (ETC). On the interior regions of the microspheres, sensor array platform is placed where all the chemical and immunological reactions are performed. These microspheres are located on the inverted pyramidal microchambers of microchip. A Charge-Coupled Device (CCD) video chip visualizes and captures the various optical signals generated by the reactions on the microspheres. The ETC system has the advantage over the ELISA in having porous beads, which allows greater number of antibody molecules to capture and thus detect, CRP at extremely low concentrations. In ELISA, antigen–antibody interactions are generated on a single layer at the bottom of the well [14].

c. OraQuick: To expedite screening and accurately diagnose HIV infection, rapid POC HIV tests have been developed[15], which provides results in 20 minutes. The fluid to be diagnosed is mixed in a vial with developing solution and the results are displayed on a testing device. It is a stick-like device with a fabric swab on one end which is inserted into a tube of testing fluid[7]. OraQuick® is the first FDA-approved oral swab in-home test for HIV-1 and HIV-2.

d. Integrated microfluidic platform for oral diagnostics (IMPOD): IMPOD, a POC diagnostic test, helps in the rapid quantification of salivary biomarkers related to oral disease. It facilitates hands-free saliva analysis by integrating sample pretreatment with electrophoretic immunoassays to quickly measure analyte concentrations in minimally pretreated saliva samples. Rapid measurement of levels of the collagen cleaving enzyme MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved. The hand-held IMPOD has been used to rapidly (3–10 minutes) measure the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva[7].

Concluding Remarks and Future Perspectives

This review summarizes periodontitis-associated biomarkers, traditional clinical methods, and recently developed POCT platforms (LOC and paper-based platforms, chairside tests, and wearable devices) for diagnosing periodontitis. Some of these (e.g., chairside tests) have already been used clinically. Recent developments in POC periodontitis testing mostly derive from the development of nanotechnology: the exploration and use of nanomaterials bring improved sensitivity and decreased reaction times and detection costs to POC testing[16, 17]. In addition, multianalyte detection is a powerful tool for increasing the accuracy of periodontitis diagnosis[18]. Multianalyte detection can furthermore reduce the demand for sample volume compared with multiple individual tests, and thus is especially suitable for periodontitis diagnostics because the available volume of GCF is very limited (normally less than 2 ml). However, several challenges remain to be addressed for periodontitis POCT platforms.

For the future development and applications of POC periodontitis testing, researchers and clinicians should concentrate their efforts on three aspects of upstream and downstream periodontitis diagnostics. First, standard collection and treatment protocol for samples should be established such that detection results can be easily compared within the same evaluation system. Second, to develop periodontitis POCT platforms, two different directions could be explored: prevention and prognosis. For periodontitis prevention, simple and convenient qualitative or semi-quantitative readout POCT methods (e.g., colorimetric detection) are preferred. For periodontitis prognosis, POCT platforms based on quantitative methods, such as fluorescence and

electrochemical methods are suggested because they provide more accurate results [19, 20–23]. Third, to analyse downstream results, long-distance medicine can be established via a mobile app or website to help patients consult with doctors. In addition, a personal record file for every user that contains their detection results can be set up to track their disease history.

By collecting diagnostic and therapeutic information (involving outpatient diagnostics results, therapeutic regimens, and prognosis results) from all periodontitis patients, a cloud database about periodontitis therapy can be constructed. In combination with machine learning and big-data analytics, more accurate diagnosis and more effective treatments could be provided to users in the future (Figure 3).

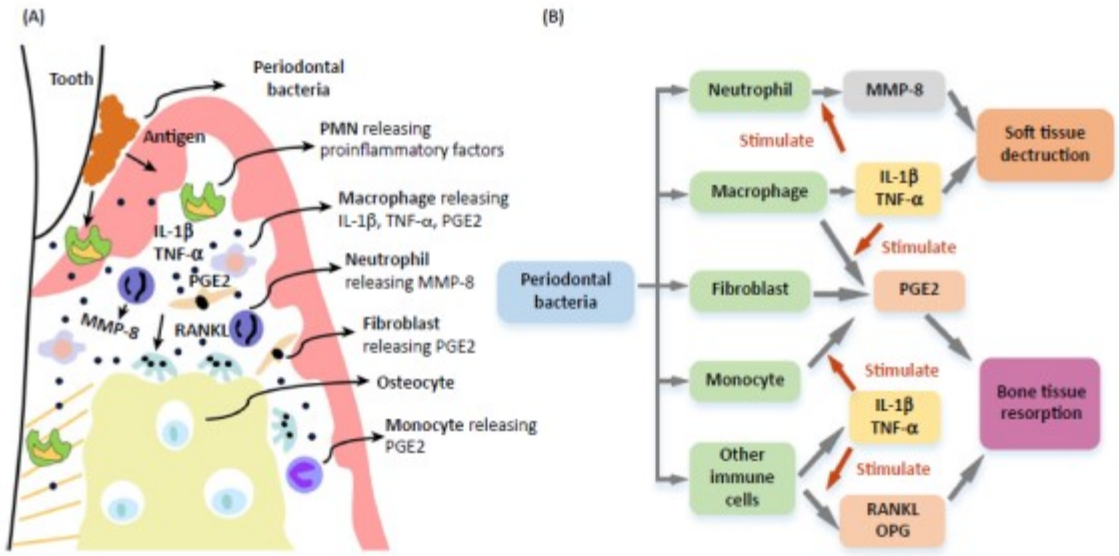

Figure 1. Pathogenesis and Associated Biomarkers of Human Periodontitis

(A) Physiological structure and (B) schematic of the response of activated immune cells [e.g., Polymorphonuclear leukocytes (PMNs), monocytes macrophages, fibroblasts, etc.] to periodontal pathogens and released proinflammatory cytokines and proteases (e.g., IL-1b, TNF-a, MMPs).

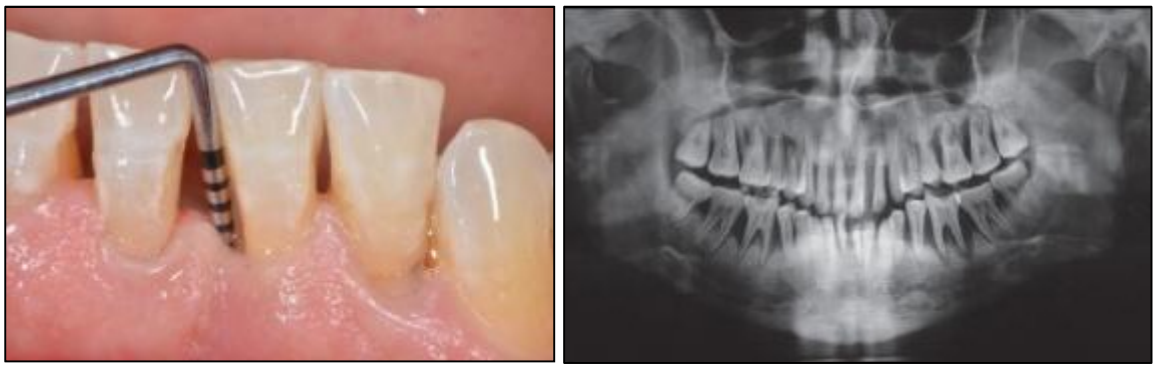

Figure 2: Traditional methods of periodontal examination

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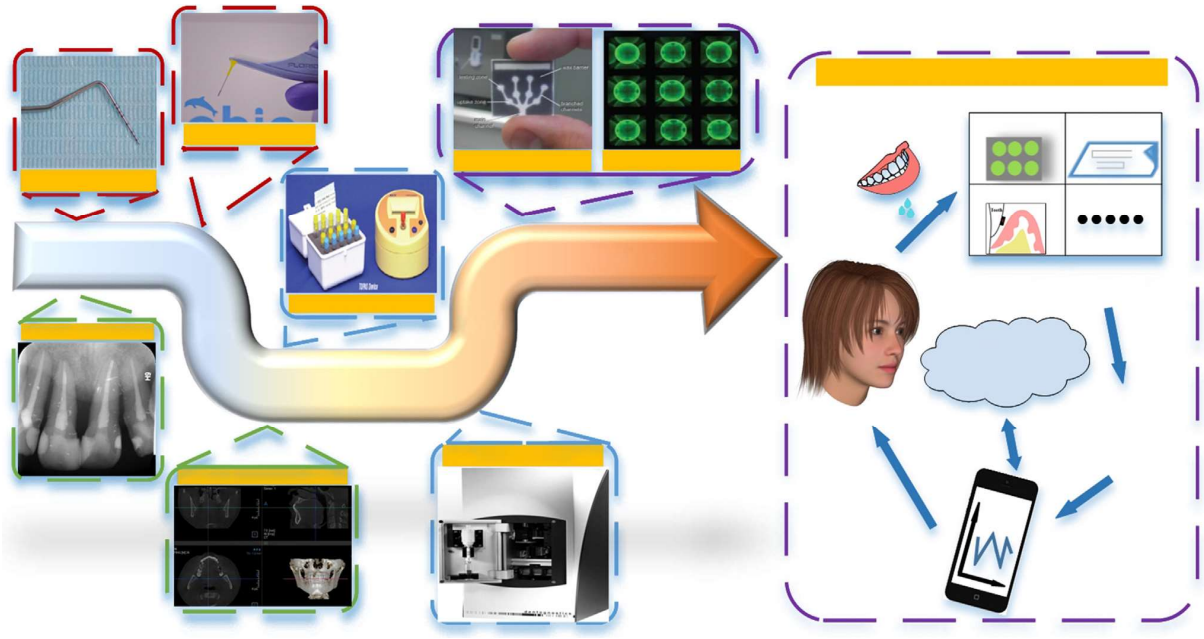

Figure 3. A Roadmap of the Developing History of Periodontitis Diagnostics and Future Perspectives for POCT Strategies.[23] Traditional clinical diagnostic methods (e.g., probing and radiography systems) are the basis of periodontitis diagnostics, and these first appeared in the 1980s.POCT platforms (e.g., chairside system and test kit, LOC, and paper-based devices) arose in the 1990s and are now beginning to be used in the clinic. Integrated POCT systems (including POCT platform, smartphone readout, and big-data analysis) are envisaged as a routine way to efficiently and accurately diagnose periodontitis in the future. Abbreviations: CBCT, cone beam computer tomography; LOC, lab-on-a-chip; POCT, point-of-care testing.

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