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SALIVA AS A DIAGNOSTIC TOOL: THE FUTURE OF NON-INVASIVE DISEASE DETECTION

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ABSTRACT

Saliva is an easily accessible biological fluid that has gained increasing attention as a potential diagnostic specimen. In addition to its well-established roles in lubrication, digestion, taste and protection of the oral cavity, saliva contains proteins, enzymes, hormones, antibodies, nucleic acids, metabolites and microbial components that may reflect physiological and pathological changes. These characteristics have encouraged investigation of saliva as a non-invasive source of biomarkers for both oral and systemic diseases. Salivary diagnostics has been explored in endocrine disorders, cardiovascular diseases, diabetes mellitus, infectious diseases, malignancies and periodontal disease. The presence of cell-free DNA, RNA, microRNA, proteins, metabolites and extracellular vesicles has also led to the concept of saliva as a potential liquid-biopsy medium. Its painless collection and suitability for repeated sampling make it particularly attractive for screening and point-of-care applications. This narrative review discusses the composition and physiological functions of saliva and summarizes its emerging diagnostic applications in cardiovascular disease, endocrine disorders, oral cancer, diabetes mellitus, dental caries and periodontal disease. Although numerous biomarkers have demonstrated promising associations with disease, differences in sample collection, salivary flow, oral health, medications, diet and analytical methods remain important limitations. Further prospective studies, standardized protocols and validated diagnostic thresholds are necessary before most salivary biomarkers can be incorporated into routine clinical practice. With continued advances in molecular diagnostics, biosensors and point-of-care technologies, saliva may become an important component of non-invasive and personalized healthcare.

Keywords: Saliva; salivary biomarkers; liquid biopsy; oral cancer; systemic diseases; non-invasive diagnosis; periodontal disease.

INTRODUCTION

Saliva is a complex biological secretion with an important role in maintaining oral health. It lubricates the oral tissues, assists swallowing and digestion, contributes to taste perception, protects teeth and mucosa, and participates in antimicrobial defence. Its composition includes water, electrolytes, proteins, enzymes, immunoglobulins, nucleic acids, metabolites and microbial components.

The diagnostic importance of saliva arises from the fact that many of these constituents change in response to physiological or pathological processes. Consequently, saliva has increasingly been investigated as a source of biomarkers that may provide information about oral and systemic health.

The concept of liquid biopsy has further expanded interest in salivary diagnostics. Unlike conventional tissue biopsy, which requires removal of tissue, liquid biopsy examines disease-associated molecular material present in biological fluids. Depending on the disease, this material may include cell-free DNA, RNA, proteins, metabolites and extracellular vesicles. Saliva contains several of these molecular components and may therefore provide a convenient source for non-invasive biomarker analysis.

Compared with venous blood collection, saliva sampling is generally painless, simple and suitable for repeated collection. These characteristics make it particularly attractive for population screening, home-based sampling and point-of-care testing. Nevertheless, salivary biomarker concentrations can be influenced by factors such as salivary flow, circadian rhythm, food intake, hydration, medications and oral disease. Standardization is therefore essential before salivary tests can be reliably incorporated into routine clinical practice.

This review summarizes the biological basis of salivary diagnostics and discusses its potential applications in selected oral and systemic diseases.

COMPOSITION OF SALIVA

Saliva is predominantly water, with the remaining fraction consisting of inorganic ions and a wide range of organic molecules.

Inorganic components

Important inorganic constituents include:

- Sodium
- Potassium
- Calcium
- Chloride
- Bicarbonate
- Phosphate
- Fluoride
- Magnesium

These components contribute to buffering, maintenance of oral pH, tooth mineralization and overall oral homeostasis.

Organic components

Saliva contains numerous proteins and enzymes, including:

- α -amylase

- Lysozyme
- Lactoferrin
- Peroxidases
- Mucins
- Histatins
- Proline-rich proteins
- Antimicrobial peptides

Immunological components include secretory immunoglobulin A, immunoglobulin G, immunoglobulin M, cytokines and other inflammatory mediators. Nucleic acids such as DNA, messenger RNA and microRNA can also be detected in saliva. In addition, metabolites such as glucose, urea, uric acid, creatinine and lactate contribute to the salivary molecular profile.

Microbial components

Saliva contains microorganisms and microbial products originating largely from the oral environment. Bacterial, viral and fungal genetic material may therefore be detected and investigated for diagnostic purposes.

Salivary glands and secretion

The major salivary glands are the parotid, submandibular and sublingual glands, while numerous minor glands are distributed throughout the oral mucosa. The major glands contribute most of the resting and stimulated salivary secretion, although their relative contribution varies with the degree and type of stimulation.

Salivary secretion is regulated primarily by the autonomic nervous system. Parasympathetic and sympathetic stimulation influences salivary flow and composition. Mechanical stimulation during mastication, as well as sensory and olfactory stimuli, can further increase salivary secretion.

PHYSIOLOGICAL FUNCTIONS OF SALIVA

Immunological and antimicrobial functions

Saliva forms an important component of the first line of defence within the oral cavity. It contains several antimicrobial substances that help regulate microbial colonization and protect oral tissues.

Lysozyme acts on bacterial cell-wall peptidoglycan and contributes to bacterial destruction.

Lactoferrin binds iron and can restrict its availability to microorganisms.

Histatins have notable antifungal activity, particularly against *Candida* species.

Defensins are antimicrobial peptides capable of interfering with microbial membranes.

Mucins and other salivary glycoproteins form a protective layer over oral surfaces. They can interfere with microbial adhesion, trap microorganisms and facilitate their removal through swallowing.

Lubrication

The water content and mucin-rich composition of saliva provide effective lubrication of the

oral cavity. This facilitates mastication, swallowing and speech while reducing friction between food and the oral mucosa.

Saliva also helps maintain mucosal hydration and protects oral tissues from mechanical irritation.

Taste and digestive functions

Saliva is essential for normal taste perception because taste-producing molecules need to dissolve before interacting with taste receptors. It also helps remove food debris and maintains an aqueous environment around the taste buds.

Digestion begins in the oral cavity, with salivary α -amylase initiating the breakdown of dietary starch. Saliva also assists in transforming chewed food into a cohesive bolus that can be swallowed efficiently.

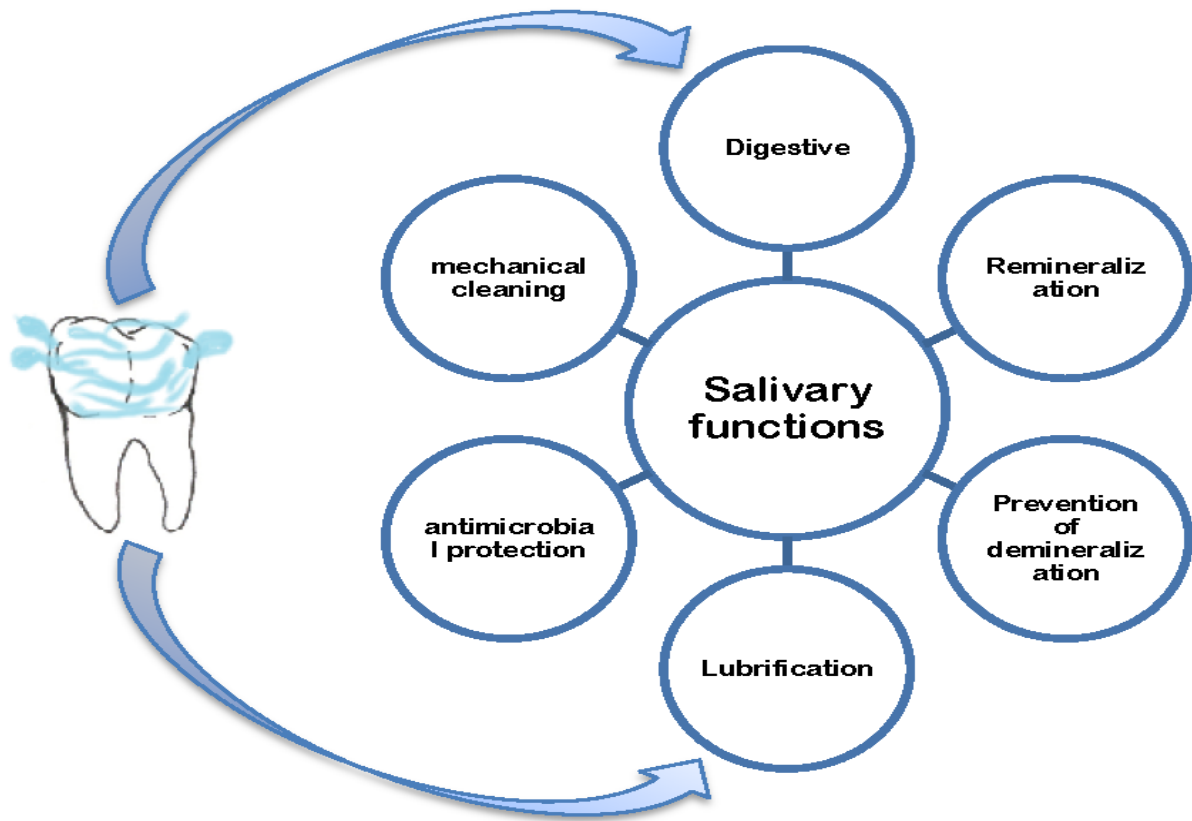
Salivary proteome and oral homeostasis

The salivary proteome represents the collection of proteins present in saliva and reflects a dynamic interaction between the salivary glands, oral tissues, microorganisms and systemic influences.

Salivary proteins contribute to:

- Lubrication and tissue protection
- Antimicrobial defence
- Immune regulation
- Digestion
- Maintenance of tooth integrity
- Enamel pellicle formation
- Wound healing and tissue repair
- Taste modulation

Saliva also contributes to tooth protection by supplying calcium and phosphate and by helping maintain conditions favourable for enamel remineralization.



SALIVA AS A DIAGNOSTIC TOOL

The diagnostic potential of saliva is based on its diverse molecular composition. Alterations in salivary proteins, nucleic acids, hormones, metabolites and microbial components may provide information about disease processes.

Salivary biomarkers have been investigated in endocrine disorders, cardiovascular disease, diabetes mellitus, cancer, infectious diseases and periodontal disease. However, a molecule being detectable in saliva does not automatically make it a clinically useful biomarker. Diagnostic

performance must be established through appropriately designed studies evaluating sensitivity, specificity, reproducibility and clinical relevance.

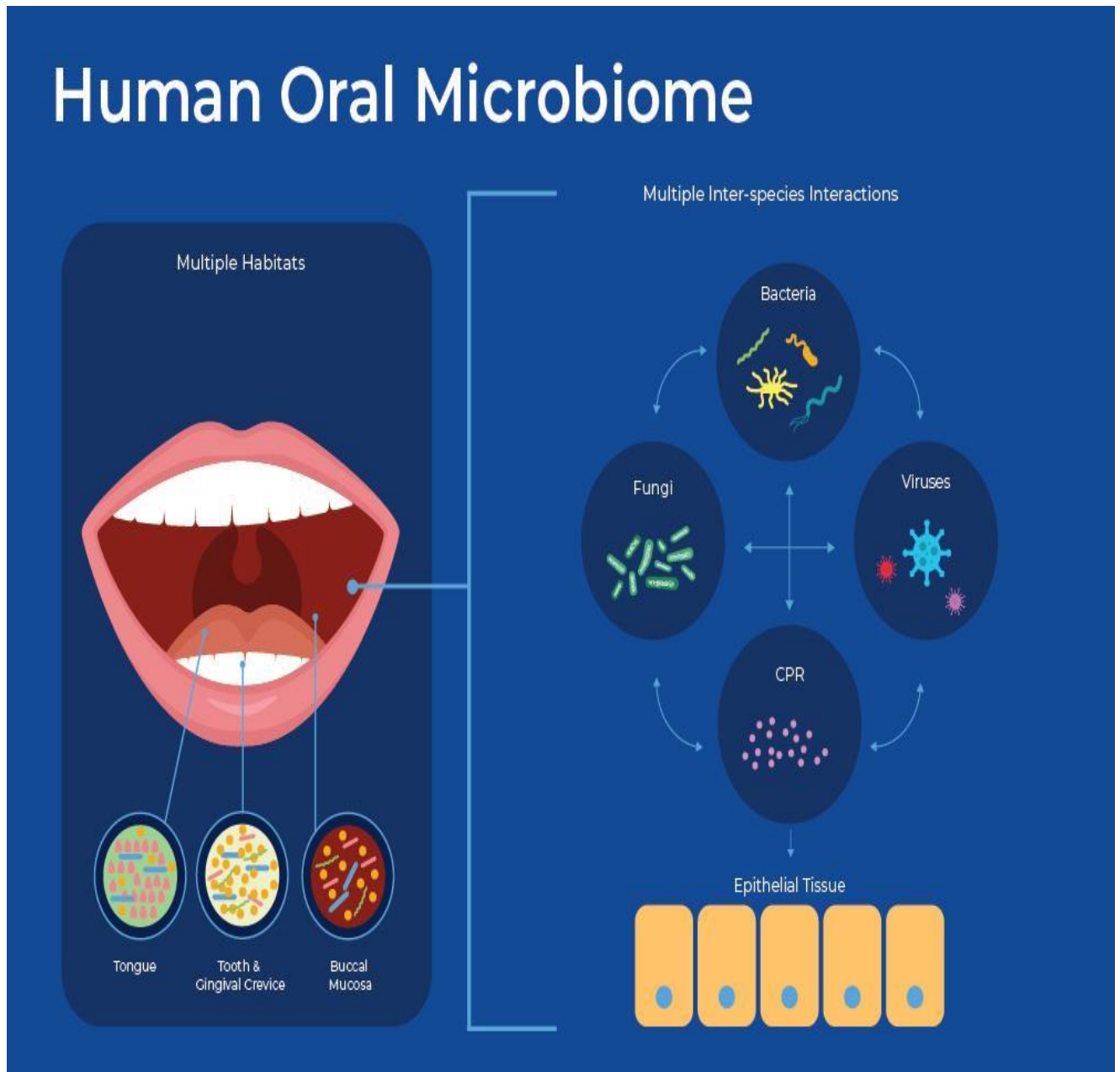
Potential advantages of salivary testing

Salivary diagnostics offers several practical advantages:

- Non-invasive collection
- Minimal patient discomfort
- Simple sample acquisition
- Potential for repeated sampling
- Potentially lower collection costs
- Suitability for home-based sampling
- Potential use in point-of-care devices
- Reduced dependence on venipuncture
- Potential application in population screening
- Compatibility with personalized and preventive healthcare

However, these advantages should not be interpreted as evidence that saliva can currently

replace blood or tissue-based diagnostic methods.



MECHANISM OF SALIVARY BIOMARKER DETECTION

The biological pathway by which a systemic biomarker reaches saliva differs according to the molecule. Some substances may diffuse from the circulation, while others may originate from oral tissues, immune cells, microorganisms or salivary glands. Therefore, interpretation of a salivary biomarker requires consideration of both local and systemic sources.

CARDIOVASCULAR DISEASE

Cardiovascular disease is an important area of research in salivary diagnostics because conventional cardiac biomarkers are generally measured in blood. Researchers have therefore investigated whether cardiac and inflammatory markers can also be identified in saliva.

Potential salivary biomarkers that have been investigated include:

- Cardiac troponin I
- Creatine kinase-MB
- Myoglobin
- C-reactive protein
- Myeloperoxidase
- Matrix metalloproteinase-9
- Brain natriuretic peptide

A systematic review identified 40 salivary biomarkers investigated in cardiovascular disease. CRP, CK-MB, MMP-9, troponin I, myeloperoxidase, myoglobin and BNP were among the most frequently studied. Salivary CRP showed the most consistent association with cardiovascular disease, although evidence for several other markers remained inconsistent.

Acute myocardial infarction

Acute myocardial infarction requires rapid recognition and treatment. This has stimulated research into whether markers of myocardial injury, particularly cardiac troponins, can be detected in saliva.

Although preliminary findings suggest potential diagnostic value, current evidence is insufficient to establish salivary testing as a replacement for established serum cardiac biomarkers. Variations in assay techniques, study populations and biomarker concentrations remain significant limitations.

Saliva may therefore have future applications as a complementary screening or point-of-care specimen, but further validation is required before it can be considered a routine diagnostic alternative.

ENDOCRINE DISEASES

Endocrine disorders represent one of the more established areas of salivary biomarker research. Several steroid hormones can be measured in saliva, including:

- Cortisol
- Cortisone
- Testosterone
- Progesterone
- Estradiol
- Dehydroepiandrosterone

Because certain steroid hormones can enter saliva from the circulation, salivary measurements may provide information about their biologically available fraction.

Cushing syndrome

Late-night salivary cortisol is one of the best-established clinical applications of salivary hormone testing.

Under normal physiological conditions, cortisol follows a circadian pattern, with concentrations highest in the morning and lowest around midnight. In Cushing syndrome, this normal decline is lost.

The Endocrine Society includes late-night salivary cortisol among the recommended initial tests for suspected Cushing syndrome, with two measurements recommended in the diagnostic evaluation.

One of the practical advantages is that samples can be collected at home, reducing the influence of hospital-related stress and allowing measurement close to the patient's usual bedtime.

However, interpretation requires appropriate clinical context because factors such as medications, sleep pattern, shift work and certain medical conditions can affect cortisol measurements.

ONCOLOGY

Cancer is one of the most extensively investigated areas of salivary diagnostics. Saliva contains several molecular components that may reflect tumour-associated changes, including:

- Cell-free DNA
- RNA
- MicroRNA
- Proteins
- Cytokines
- Extracellular vesicles
- Metabolites

These molecules may potentially provide a molecular signature associated with tumour development or progression.

Oral squamous cell carcinoma

The diagnostic potential of saliva is particularly relevant to oral squamous cell carcinoma (OSCC) because the disease develops within the oral and oropharyngeal environment.

Investigated salivary biomarkers include:

- IL-1 β
- IL-6
- IL-8
- TNF- α
- MMP-9
- Cell-free DNA
- Messenger RNA
- MicroRNA
- Salivary metabolites

Systematic reviews have reported increased salivary concentrations of several inflammatory cytokines, particularly IL-6, IL-8, TNF- α and IL-1 β , among patients with oral cancer.

MicroRNAs and messenger RNA have also received considerable attention. Several studies have demonstrated potentially useful diagnostic performance, particularly when molecular markers are measured using molecular amplification techniques.

Nevertheless, these findings should be interpreted cautiously. Oral cancer diagnosis still depends on clinical assessment and histopathological examination. Salivary biomarkers currently represent promising adjunctive or screening tools, rather than replacements for tissue

biopsy.

DIABETES MELLITUS

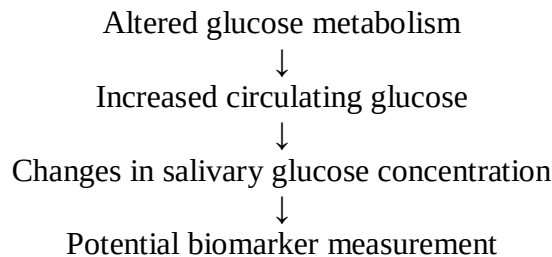
Diabetes mellitus is another systemic condition in which salivary biomarkers have been extensively investigated.

Potential salivary markers include:

- Glucose
- Fructosamine
- 1,5-Anhydroglucitol
- Glycated proteins
- α -Amylase
- Immunoglobulins
- Oxidative stress markers
- Inflammatory mediators

Salivary glucose has received particular attention because its measurement could potentially provide a painless method for screening or monitoring glucose metabolism.

The proposed relationship can be summarized as:



A systematic review and meta-analysis found higher salivary glucose concentrations in individuals with type 2 diabetes and reported an overall association between salivary glucose, blood glucose and HbA1c.

More recent evidence continues to investigate this relationship, while emphasizing substantial methodological heterogeneity between studies.¹⁶ Therefore, salivary glucose currently appears more appropriate as a potential complementary or screening biomarker than as a replacement for established blood-based testing.

ORAL DISEASES

Dental caries

Dental caries is a multifactorial disease characterized by an imbalance between demineralization and remineralization of dental hard tissues. Microbial metabolism produces acids that contribute to mineral loss when the oral environment remains favourable to demineralization.

Saliva provides several protective mechanisms against caries. It:

- Buffers acids
- Helps clear food debris and microorganisms

- Supplies calcium and phosphate
- Supports enamel remineralization
- Contains antimicrobial molecules
- Maintains oral fluid balance

Changes in salivary flow and composition have therefore been investigated in relation to caries susceptibility and activity. However, no single salivary parameter has demonstrated sufficient diagnostic reliability to independently establish the presence of dental caries.

Salivary testing may therefore have greater value in assessing caries risk and oral environment than in replacing conventional clinical and radiographic diagnosis.

PERIODONTAL DISEASE

Periodontal disease involves inflammatory destruction of the tissues supporting the teeth and is strongly associated with dysbiotic dental biofilm and an altered host immune response.

During periodontal inflammation, microorganisms, inflammatory cells and damaged periodontal tissues contribute molecules to gingival crevicular fluid and saliva. These changes have stimulated extensive research into salivary biomarkers for periodontal disease.

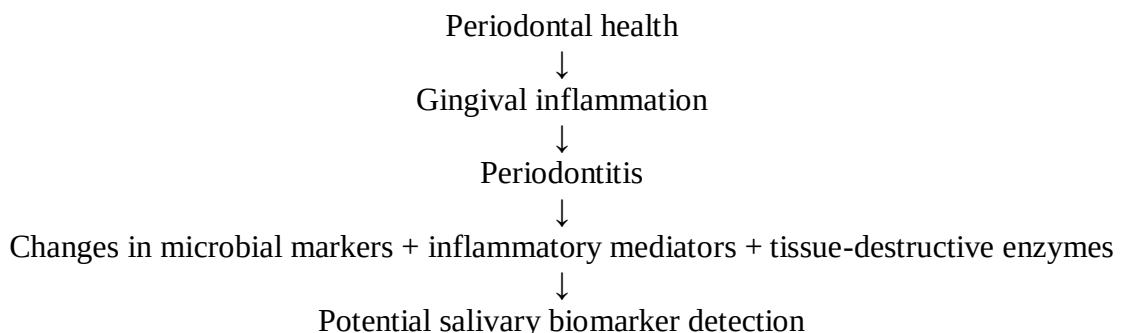
Potential biomarkers include:

- Matrix metalloproteinases
- Interleukins
- Tumour necrosis factor- α
- Lactoferrin
- Immunoglobulins
- Complement proteins
- Microbial markers
- Other host-response proteins

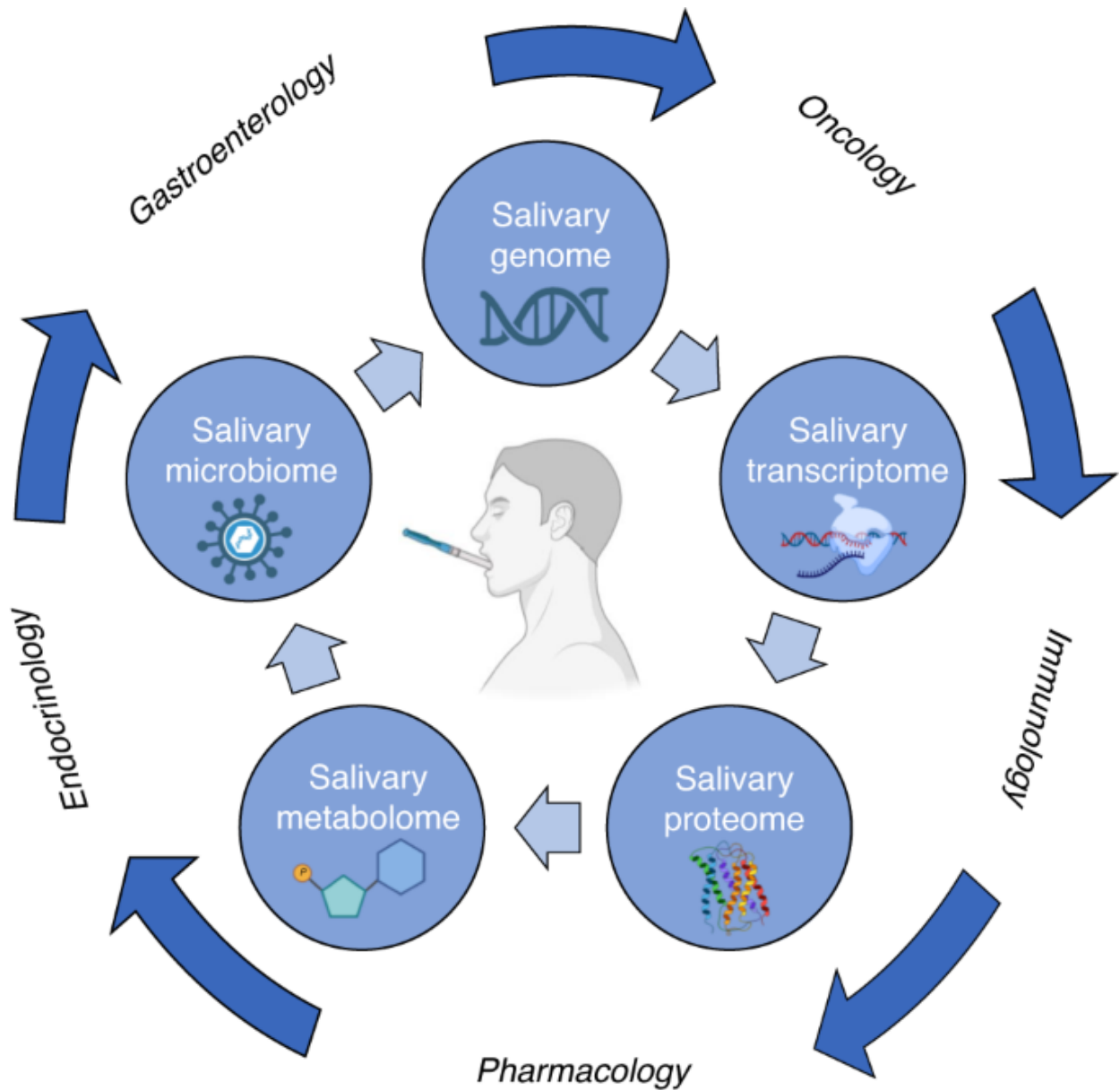
Recent systematic reviews have reported associations between salivary MMP-8 and periodontal disease, while salivary proteomic studies have identified several proteins that differ between periodontally healthy individuals and patients with periodontitis.

Salivary aMMP-8 point-of-care testing has also been investigated for periodontal disease detection, suggesting potential utility in screening and risk assessment.

The concept can be represented as:



Despite promising results, salivary testing should currently complement rather than replace periodontal examination, probing measurements and radiographic assessment.



LIMITATIONS OF SALIVARY DIAGNOSTICS

Despite its potential, several factors can influence salivary biomarker measurements.

Important sources of variability include:

- Salivary flow rate
- Circadian rhythm
- Recent food and beverage intake
- Hydration status
- Medications
- Age
- Smoking and other exposures
- Oral hygiene
- Periodontal and other oral diseases
- Method of sample collection
- Sample storage

- Laboratory assay techniques

Variation in these factors can make it difficult to establish universal reference ranges and diagnostic thresholds.

Another major challenge is distinguishing biomarkers originating from systemic disease from those produced locally within the oral cavity. This is particularly important in patients with periodontal inflammation, oral infections or other local conditions.

Consequently, a promising biomarker identified in a research study cannot automatically be considered a clinically validated diagnostic test.

FUTURE PERSPECTIVES

The future development of salivary diagnostics will depend on improvements in biomarker validation, analytical sensitivity and standardization.

Emerging technologies such as:

- Microfluidic devices
- Biosensors
- Salivary proteomics
- Genomics
- Transcriptomics
- Metabolomics
- Next-generation sequencing
- Artificial intelligence

may allow simultaneous analysis of multiple biomarkers from very small saliva samples.

Portable point-of-care platforms could eventually permit testing in dental clinics, primary healthcare centres and home settings. Such systems may be particularly useful for screening populations where conventional blood sampling is difficult or impractical.

However, large prospective studies and standardized protocols remain necessary. Future research should focus not only on identifying new biomarkers but also on determining whether their measurement actually improves clinical decision-making and patient outcomes.

CONCLUSION

Saliva has evolved from being regarded primarily as a fluid responsible for lubrication and oral protection to a promising source of diagnostic information. Its complex molecular composition enables investigation of proteins, nucleic acids, hormones, metabolites and microbial components associated with both oral and systemic disease.

Its major advantages include painless collection, ease of repeated sampling and potential compatibility with point-of-care technologies. Research has demonstrated encouraging applications in endocrine disorders, cardiovascular disease, diabetes mellitus, oral cancer and periodontal disease.

Nevertheless, most salivary biomarkers remain at the research or validation stage. Variations in collection methods, oral health, biomarker concentrations and analytical procedures continue to limit their widespread clinical application.

The future of salivary diagnostics therefore lies not simply in replacing blood or tissue biopsy,

but in developing reliable complementary, screening and monitoring tools. With appropriate validation and technological advancement, saliva may become an important component of preventive, personalized and minimally invasive healthcare.

From protecting the oral cavity to providing molecular clues about systemic health, saliva may ultimately serve as a diagnostic window into the human body.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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