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Emerging technologies for salivaomics in cancer detection

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Abstract

Salivary diagnostics has great potential to be used in the early detection and prevention of many cancerous diseases. If implemented with rigour and efficiency, it can result in improving patient survival times and achieving earlier diagnosis of disease. Recently, extraordinary efforts have been taken to develop non-invasive technologies that can be applied without complicated and expensive procedures. Saliva is a biofluid that has demonstrated excellent properties and can be used as a diagnostic fluid, since many of the biomarkers suggested for cancers can also be found in whole saliva, apart from blood or other body fluids. The currently accepted gold standard methods for biomarker development include chromatography, mass spectometry, gel electrophoresis, microarrays and polymerase chain reaction-based quantification. However, salivary diagnostics is a flourishing field with the rapid development of novel technologies associated with point-of-care diagnostics, RNA sequencing, electrochemical detection and liquid biopsy. Those technologies will help introduce population-based screening programs, thus enabling early detection, prognosis assessment and disease monitoring. The purpose of this review is to give a comprehensive update on the emerging diagnostic technologies and tools for the early detection of cancerous diseases based on saliva.

Keywords: salivary diagnostics ● cancer ● RNA-Sequencing ● point-of-care ● liquid biopsy

Introduction

Saliva is a complex fluid that is composed of water, cells, debris, organic and inorganic molecules that may reflect the physiological state of an individual condition, since many of the componenets of the saliva also play an important role in processes taking part in distal portions of the body [1]. Currently, approximately 40% of markers suggested for diseases such as cancer, cardiovascular disease and stroke can also be found in whole saliva [2]. A biomarker can be defined as a measurable and quantifiable biological parameter that can serve either as an indicator for health, disease status, environmental exposure or pharmacological responses to a therapeutic intervention [3]. Prognostic biomarkers are used as indicators of a benign or a malignant condition, whereas diagnostic biomarkers show the development of a cancer [4].

Siegel *et al.* reported that 1.7 million Americans are diagnosed with cancer every year and over 500,000 individuals do not survive the disease [5]. Hence, a lot of efforts have been done to advance the field of salivary diagnostic technology, which is likely to revolutionize the way cancerous diseases will be diagnosed in the future [6].

To successfully translate research on salivary biomarkers to the chairside, biomarker studies should follow the principles laid out in the prospective-specimen-collection, retrospective-blinded evaluation

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(PRoBE design). In this approach, samples are first collected prospectively from a cohort of target population prior to diagnosis. After examination of the patient, individuals with known diagnosis and control subjects will be selected randomly from the cohort and their specimens will be tested in a blinded study design [7]. If applied with rigour and appropriate sampling of patient population, definitive validation of identified biomarkers can result in the Food and Drug Administration regulatory approval.

Despite the acceptance of salivary diagnostics for the detection of cancerous diseases, the absence of a mechanistic rationale in regards to the transmission of biomarkers between the distal tumour and the oral cavity poses a risk that has the potential of undermining saliva's value for the detection of tumour diseases. However, recent studies focused on exosome secretion and biogenesis have attempted to unravel this phenomenon [8–10].

Emerging technologies for salivary diagnostics of cancer

Point-of-care diagnostics

Point-of-care (POC) technologies are newly emerging methods, that when used in conjunction with biomarker identification, have the potential to be used in screening and non-invasive diagnostics in a rapid and convenient fashion [11]. Current diagnostic methods used for the detection of malignant cancers have also significant limitations such as low sensitivity and low specificity. They are time-consuming, invasive, cost-prohibitive, and complex to perform [12, 13]. In addition, the long assay time may cause degradation of many important constituents in the patient samples before quantification can be made [14, 15]. Therefore, before entering the clinical settings, these POC tests should be appropriately prepared *i.e.* to prove their validity, reliability, reproducibility and robustness [16].

There are several newly emerging technologies that integrate salivary diagnostics with microfluidics or micro/nanoelectromechanical systems (MEMS/NEMS). Microfluidics consist of manipulation of liquids at the microscale to miniaturize and automate many techniques that may normally require trained personnel with traditional laboratory equipment. MEMS/NEMS devices are composed of mechanical elements, sensors, actuators and electronics on a common silicon substrate developer through microfabrication technology. Those technologies enable users to measure proteins, DNA, transcripts (mRNA), electrolytes and small molecules in saliva [17, 18]. Currently developed MEMS/NEMS platforms use a variety of techniques to perform detection, including electrochemical sensing [19], on-chip reverse transcription polymerase chain reaction (RT-PCR) [20], microsphere-based optical fibre array [21], high-throughput DNA microarray, surface plasmon resonance optical system [22] or microchip electrophoretic immunoassay [23].

Mishra *et al.* divided currently used technologies and devices according to the type of biomarker and cancer, as follows [13]. The biomarker types are:

- Transcriptomic biomarkers: breast cancer (nanographene oxide-polyethylene glycol methyl ether methacrylate with DNase 1 to detect microRNA-10b and microRNA-10a) [24], oral cancer (electrical controlled magnetic EC Sensor to detect microRNA-200a) [25], prostate cancer [nano-graphene oxide (nGO)/FAM-anti-miR-21 to detect microRNA-21 and nGO/Cy5-anti-miR-141 to detect microRNA-141] [26];
- Genomic biomarkers: oral cancer [electrochemical sensor using endonuclease target recycling amplification to detect oral cancer overexpressed 1 (ORAOV1) [27] or electric field-induced release and measurement method for detection of epidermal growth factor receptor (EGFR) [28]];
- Metabolomic biomarkers: oral cancer (wireless mouthguard ezymatic biosensor to detect uric acid [29] or lactic acid [30]), gastric cancer (microfluidic optoelectronic [31] or graphene based-antimicrobial peptides with passive detection of Helicobacter pylori [32]);
- Proteomic biomarkers: liver cancer (surface enhanced Raman spectroscopy using optical nanoanntenas functionalized with aptamers for detection of MnSOD) [33], breast cancer [surface plasma resonance biosensor based on Au/ZnO thin films for carcinoma antigen 15-3 (CA15-3)] [34];
- Multiplex: silicon nanowire field effect transistor for IL-8 and TNF-α [35].

The newly developed POC tests for 'lab-on-a-chip' allows detection of multiple biomarkers, thus facilitating the diagnosis of many human diseases at the same time [36].

In 2003, the University of California at Los Angeles (UCLA) Collaborative Oral Fluid Diagnostic Research Center was established with the major aim of developing the platform for using nanotechnology and microtechnology for detection of salivary proteins and genomic biomarkers. An integrated POC electrochemical multiplexing saliva-based platform for oral cancer detection emerged [17, 37]. This platform can detect both salivary proteins and nucleic acids as well as measure up to eight different biomarkers in a single test in less than 15 min. under ambient conditions. The salivary test in an Indian cohort of oral cancer saliva samples achieved 90% sensitivity and 90% specificity for both interleukin 8 (IL-8) and IL-8 protein messenger RNA (mRNA) [38]. This method can potentially be used for screening and assessment of the risk for oral cancer, as well as identification of patients that may need a biopsy [37]. Another saliva-based molecular test, OraRisk® human papilloma virus (HPV) with Reflex (Quest Diagnostics, Los Angeles, CA, USA) can determine the presence of HPV types associated with a high risk of developing oral cancer.

Emerging novel POC techniques used specifically for oncogenic mutation detection in clinical practice include: gold nanoparticle-based mutation capture and naked-eye visualization for the detection of single nucleotide polymorphism mutation [39, 40] or a combination of magnetic and gold nanoparticle methods to identify KRAS gene mutations [41]. Another method – microfluidic platforms combine genetic analysis with microfluidic systems [40, 42], for example, an integrated microfluidic system for JAK2-V617F mutation detection, present in various hematological malignancies [43].

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Along with these recent scientific advancements, there is an emerging need to move salivary diagnostics out of the research lab into clinical practice. Point-of-care technologies can provide non-invasive, rapid, easy and accurate measurements directly from saliva for monitoring different medical conditions including cancers [37].

RNA sequencing

RNA-Sequencing (RNA-Seq) is a newly emerging high-throughput method for performing transcriptome profiling by means of deep-sequencing technologies. The transcriptome is composed of transcripts in a cell, and their quantity. It plays a crucial role for unravelling functional elements of the genome, the molecular components of cells, and also the mechanisms of normal development, physiology and pathology. While performing RNA Sequencing, RNA is converted to a library of cDNA fragments. Each molecule is sequenced, with or without amplification, resulting in the large number of reads, that are subsequently aligned either to a reference genome or to a transcriptome [44]. RNA-Seq has many advantages over the currently used DNA microarrays, *i.e.*: ability to detect transcripts and their isoforms, low background signal, increased dynamic range of expression, measurement of focal changes, splice variants, chimeric gene fusions and applicabilty to each species, *etc.* [44, 45].

Although RNA-Seq of saliva is challenging, because of factors such as the difficulty of performing RNA isolation, stabilitization, RNA library construction, *etc.* [46–49], the recent advancements in the field have resulted in the identification of various types of extracellular RNAs (exRNAs) such as: mRNAs and non-coding RNAs (ncRNAs) including microRNAs (miRNAs), piwi-interacting RNAs (piRNAs) and circular RNAs (circRNAs). Specifically, those ncRNAs are emerging regulators of oncogenesis and tumour progression. Because of their small size, they are more stable and less prone to degradation by ribonucleases (RNases) compared to mRNAs [15].

Currently, RNA Sequencing has a wide range of applications in cancer diagnostics. Our group at UCLA is in the process of developing salivary biomarkers for early detection of gastric cancer [50]. This study involves comprehensive RNA-Sequencing performed on 100 randomly selected gastric cancer saliva samples and 100 randomly selected non-gastric cancer matched control subjects. Bioinformatic analysis of RNA-Seq data has revealed various types of exRNAs in cell-free saliva, including 127–418 miRNAs, 32–109 piRNAs and 400 circRNAs, representing the first characterization of circRNAs in extracellular body fluid [50].

Aside from emerging salivary diagnostics, the use of RNA-Seq methodology in cancer diagnostics in other body fluids and tissues is very common [45, 51, 52]. For example, currently there are known splicing signatures of the three most common types of breast tumours [Triple Negative Breast Cancer (TNBC), non-TNBC and HER2-positive cancers] identified by means of RNA-Seq [51]. Also, alternative breast cancer 1 (BRCA1) transcripts have been detected in a subset of patients with breast cancer and a family history of breast and/or ovarian cancer [53]. Furthermore, diagnosis of acute myeloid leukaemia can be currently made based on the detection of genetic abnormalities such as t(8;21)(q22;q22) translocation or runt-related transcription factor 1 (RUNX1) fusion RUNX1–RUNX1T1 [52].

The recent progress in RNA-Seq technologies have a potential for exRNAs to serve as non-invasive diagnostic indicators of the disease in risk assessement, early diagnostics, prognostics and therapeutics for various diseases, including cancers and infectious diseases [45].

Liquid biopsy

Liquid biopsy is a biofluid test (of matrices such as serum, urine, saliva) that detects circulating tumour cells or circulating cell-free tumour DNA (ctDNA) shed into the bloodstream by cancer cells undergoing apoptosis or necrosis. Those tests are much more practical compared to genotyping of tumour tissue, which has significant limitations such as tumour heterogeneity, invasiveness, difficulties with sampling and fact that tumour tissue acquired through a biopsy reflects the condition only at the time of the examination [54–57]. In addition, tumour-associated mutations detectable in various body fluids provide the information about the early detection, assessment of molecular heterogeneity of general disease, its prognosis, recurrence, monitoring of tumour dynamics and the success or failure of systemic therapies [40, 54, 57]. Prediction of prognosis in patients with curable cancer disease can already be achieved in several tumours such as breast cancer, melanoma, ovarian or colon cancers [40, 57]. Liquid biopsy permits less invasive means of assessing the oncogenic mutation profile of a patient, and can guide the use of targeted molecular therapies resulting in an improvement of clinical outcome in oncological patients [40].

Analytical strategies to detect and quantify ctDNA in bodily fluids include next generation sequencing (NGS), PCR-based technology, digital PCR, mass spectrometry (MS), denaturing high performance liquid chromatography, peptide nucleic acid (PNA)-mediated PCR and PNA-locked nucleic acid PCR clamp, amplification refractory mutation system, beads, emulsion, amplification and magnetics (BEAMing) or pyrophosphorolysis-activated polymerization [40, 55, 56] (Table 1).

Electromagnetic field-based methods

According to the literature, the electromagnetic field can be a very useful tool in diagnosis and treatment of cancers [68–70]. Cormio *et al.* performed the study aimed to determine the diagnostic accuracy of non-invasive electromagnetic detection of bladder cancer by the tissue-resonance interaction method (TRIM-prob) with its overall sensitivity, specificity, positive and negative predictive values as well as diagnostic accuracy of 97.9%, 89.9%, 86.8%, 98.6%, and 93.6%, respectively. They concluded that TRIM-prob bladder scanning could be used to screen asymptomatic patients at high risk of developing a bladder cancer [70]. Also, surface plasmon resonance can be implemented for cancer diagnosis and photothermal therapy, in which plasmonic gold nanoparticles contribute to evoke strong electromagnetic fields on the particle surface, thus enabling tumour detection [69]. In addition, Zimmerman *et al.* have reported that intrabuccal administration of 27.12 MHz radiofrequency

Table 1 Major analytical strategies to assess oncogenic mutations from biofluid samples [40]

Analytical strategy	Oncogenic mutations	Body fluid	Tumour	Author
Molecular detection platforms for liquid biopsy				
Beads, emulsion, amplification and magnetics (BEAMing)	PIK3CA	Plasma	Colorectal cancer	Tabernero *et al.* [58]
	KRAS			
	BRAF			
Polymerase chain reaction (PCR)-based techniques				
Quantitative reverse transcription PCR (RT-qPCR)	BRAF, KRAS	Plasma	Colorectal cancer	Spindler *et al.* [59]
	KRAS	Plasma	Lung cancer	Freidin *et al.* [60]
Droplet digital PCR (ddPCR)	KRAS	Plasma	Colorectal cancer	Taly *et al.* [61]
Next generation sequencing	TP53, PIK3CA	Cell-free plasma	Breast cancer	Nakauchi *et al.* [62]
PCR enhancement techniques for liquid biopsy				
Allele specific primer amplification	KRAS, BRAF	Serum or plasma	Colorectal cancer	Thierry *et al.* [63]
Enzyme based digestion of sequences	EGFR	Lung pleural fluid	Lung cancer	Asano *et al.* [64]
Preferential homoduplex formation assay (PHFA)	1DH1	Serum, cerebrospinal fluid	Glioma	Chen *et al.* [65]
	APC	Plasma	Colorectal cancer	Diehl *et al.* [66]
Clamped-based PCR technique	EGFR	Plasma	Non-small cell lung cancer (NSCLC)	Kim *et al.* [67]

electromagnetic fields, which are amplitude-modulated at tumour-specific frequencies, results in detecting as well as blocking the growth of tumour cells in a tissue- and tumour-specific fashion in patients with various forms of cancer [68]. Interestingly, there is currently an increased interest in the use of salivary diagnostics in early detection of cancers by means of electromagnetic field, such as lung cancer [71, 72].

Electromagnetic phenomena can be highly useful for diagnostic techniques, but if applied improperly, the electromagnetic field may also cause serious harmful effects such as leukaemia [73, 74] brain tumour [74, 75], breast cancer [74, 76, 77]. This depends on the intensity of the applied electric and magnetic fields, the time of exposure as well as the nature and the frequency of changes [78, 79]. Because of the large variety of these factors, a wide spectrum of different scientific research studies and their applications are currently undertaken to elucidate the usage of electromagnetic phenomena for cancer diagnostics.

One particularly compelling branch where electric and magnetic fields are applied is in the study of exosomal vesicles (EVs), which have a diameter of approximately 30–100 nm. In case of EVs, magnetic beads proved to be very useful in various medical applications including cancer diagnostics [80, 81]. Magnetic beads coated with monoclonal antibodies directed against antigens of the specific cells can be easily separated in a magnetic field. They constitute a powerful tool for the isolation of cells, and in particular of exosomes [82]. The schemes of such cell separation in body fluids *in vivo* and *ex vivo* are presented in Figures 1 and 2, respectively.

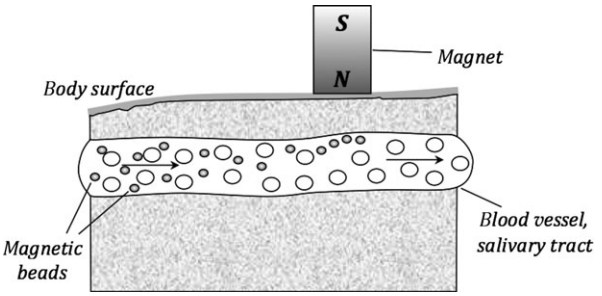

Fig. 1 The diagram shows the separation mechanism of selected cells in body fluids by means of 'magnetic beads' coated with monoclonal bodies (*in vivo*).

Electric field induced release and measurement method

The electric field induced release and measurement method (EFIRM) is a newly developed technology at UCLA. EFIRM is an electrochemical-based technique that measures the oxidation and reduction rates of a chemical reaction to perform quantification of a target biomolecule [28, 38]. This is similar to the principles used for traditional glucose metres, which measure the oxidation and reduction rates of glucose oxidase reacting with glucose.

In case of EFIRM, a capture probe that is complementary to a ctDNA target is designed and then immobilized on the surface of a

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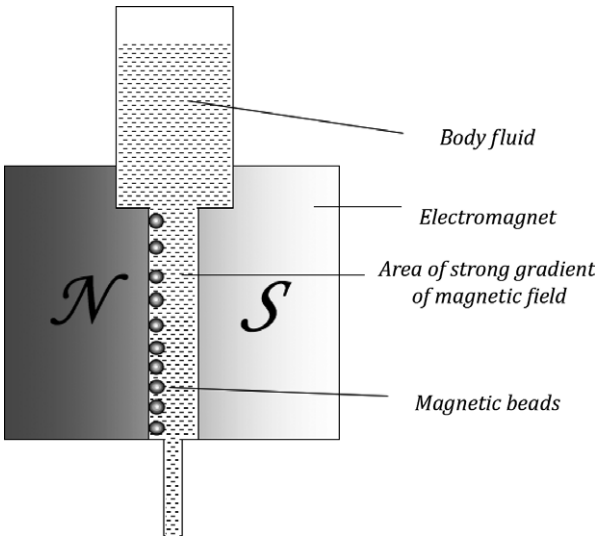

Fig. 2 Diagram of magnetic separation of selected components of body fluids using super-paramagnetic elements (*i.e.* magnetic beads) (*ex vivo*).

gold electrode by encapsulating it in a conducting polymer matrix [83]. After the immobilization of the capture probe on the surface of the electrode, the clinical specimen (*i.e.* saliva or plasma) is placed on the surface of the electrode and a cyclic square wave (CSW) is applied. This CSW is designed to specifically lyse the exosomal structure that encapsulates the ctDNA sequence and aid in the DNA hybridization process [28] (Fig. 3). Following the incubation of the target sequence to the capture probe, a detector probe that is also complementary to the ctDNA is hybridized. This detector probe has fluorescein isothiocyanate (FITC) located at its terminal end, that is then complexed to an anti-FITC antibody with horseradish peroxidase (HRP) (Fig. 4).

Finally, the quantification of the amount of the target DNA is performed by adding 3,3′,5,5′-Tetramethylbenzidine (TMB) and hydrogen peroxide readout substrate mix to the surface of the electrode. Reactions between the HRP enzyme, TMB and hydrogen peroxide will occur, and electronic circuits will be interfaced with the electrode to measure the magnitude of the reaction. If there is a large amount of ctDNA present, then, there will be a high electric current, but if no ctDNA is present, no significant amounts of electrochemical current will be measured.

The EFIRM technique was first deployed in 2009 for the examination of salivary biomarkers for oral cancer detection in a collaborative project between the UCLA School of Dentistry and UCLA School of Engineering [38]. More recent work on EFIRM with this platform on detecting ctDNA EGFR mutations in saliva has demonstrated near perfect clinical sensitivity and specificity for detection of lung cancer [38, 56, 84]. In a blinded pilot study, 40 patient saliva samples were

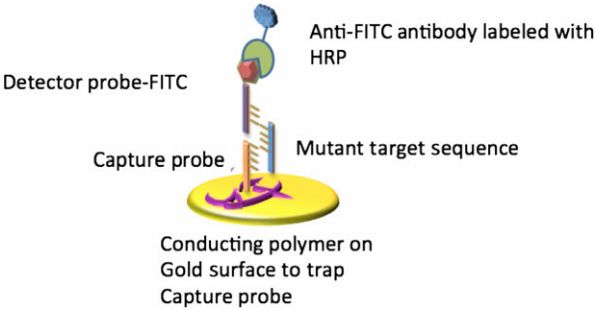

Fig. 4 Electric field–induced release and measurement (EFIRM) technology. Following the incubation of the target sequence to the capture probe, a detector probe that is also complementary to the ctDNA is hybridized. The FITC located on the terminal end of the detector probe is then complexed to an anti-FITC antibody with horseradish peroxidase (HRP).

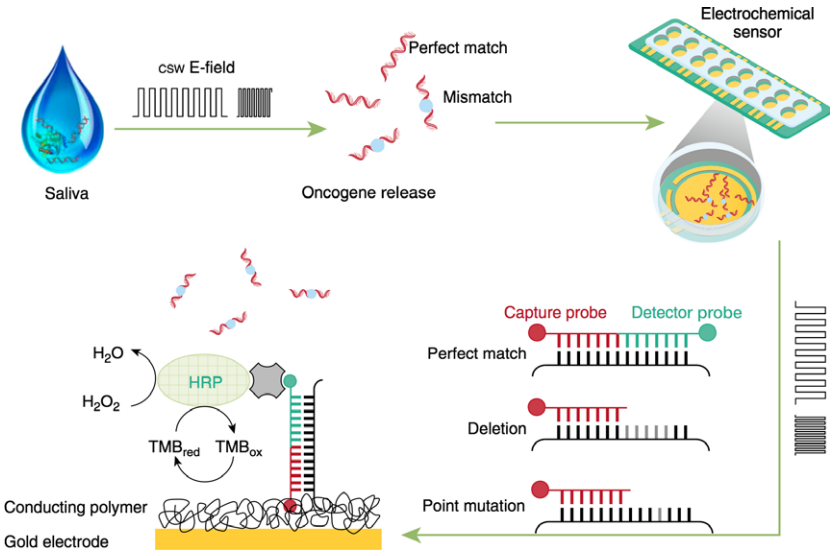

Fig. 3 Electric field–induced release and measurement (EFIRM) technology for the detection of epidermal growth factor receptor (EGFR) mutations in bodily fluids of patients with lung cancer (Reproduction from [84]).

analysed using EFIRM and compared to tissue-based oncogenic analysis. Characterizing the performance of EFIRM (area-under-the-curve of 0.94 and 0.96 was achieved for detecting exon-19 deletion and the L858R mutations, respectively). A comparison of saliva with plasma samples showed R values of 0.98 and 0.99 for the exon-19 deletion and L858R mutation, respectively [40]. Another clinical application of the method that is currently investigated is the detection of oncogenic KRAS gene mutations in patients diagnosed with pancreatic cancer [40].

Conclusions

A wide variety of emerging saliva-based technologies have already demonstrated their credibility in the early detection of many cancerous diseases. It is evident that the trend will develop towards constant improvement of POC diagnostic tools, NGS methods, advanced PCR- and electromagnetic field-based technologies as well as liquid biopsy. Further research studies will reveal which method will be the most suitable to be applied in a clinical practice.

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Conflicts of interest

David Wong is co-founder of RNAmeTRIX Inc., a molecular diagnostic company. He holds equity in RNAmeTRIX, and serves as a company Director and Scientific Advisor. The University of California also holds equity in RNAmeTRIX. Intellectual property that David Wong invented and which was patented by the University of California has been licensed to RNAmeTRIX. Additionally, he is a consultant to PeriRx. None of the other authors have a conflict of interest in relation to the study.

References

1. **Lee YH, Wong DT.** Saliva: an emerging biofluid for early detection of diseases. *Am J Dent*. 2009; 22: 241–8.
2. **Loo JA, Yan W, Ramachandran P, *et al.*** Comparative human salivary and plasma proteomes. *J Dent Res*. 2010; 89: 1016–23.
3. **Spielmann N, Wong DT.** Saliva: diagnostics and therapeutic perspectives. *Oral Dis*. 2011; 17: 345–54.
4. **Mehta S, Shelling A, Muthukaruppan A, *et al.*** Predictive and prognostic molecular markers for cancer medicine. *Ther Adv Med Oncol*. 2010; 2: 125–48.
5. **Siegel RL, Miller KD, Jemal A.** Cancer statistics, 2015. *CA Cancer J Clin*. 2015; 65: 5–29.
6. **Malamud D, Rodriguez-Chavez IR.** Saliva as a diagnostic fluid. *Dent Clin North Am*. 2011; 55: 159–78.
7. **Pepe MS, Feng Z, Janes H, *et al.*** Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. *J Natl Cancer Inst*. 2008; 100: 1432–8.
8. **Lau C, Kim Y, Chia D, *et al.*** Role of pancreatic cancer-derived exosomes in salivary biomarker development. *J Biol Chem*. 2013; 288: 26888–97.
9. **Principe S, Hui AB, Bruce J, *et al.*** Tumor-derived exosomes and microvesicles in head and neck cancer: implications for tumor biology and biomarker discovery. *Proteomics*. 2013; 13: 1608–23.
10. **Wong DT.** Salivaomics. *J Am Dent Assoc*. 2012; 143: 19S–24S.
11. **Koneru S, Tanikonda R.** Salivaomics - A promising future in early diagnosis of dental diseases. *Dent Res J (Isfahan)*. 2014; 11: 11–5.
12. **Arellano-Garcia ME, Hu S, Wang J, *et al.*** Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. *Oral Dis*. 2008; 14: 705–12.
13. **Mishra S, Saadat D, Kwon O, *et al.*** Recent advances in salivary cancer diagnostics enabled by biosensors and bioelectronics. *Biosens Bioelectron*. 2016; 81: 181–97.
14. **Barnes VM, Ciancio SG, Shibly O, *et al.*** Metabolomics reveals elevated macromolecular degradation in periodontal disease. *J Dent Res*. 2011; 90: 1293–7.
15. **Majem B, Rigau M, Reventós J, *et al.*** Non-coding RNAs in saliva: emerging biomarkers for molecular diagnostics. *Int J Mol Sci*. 2015; 16: 8676–98.
16. **Cals J, van Weert H.** Point-of-care tests in general practice: hope or hype? *Eur J Gen Pract*. 2013; 19: 251–6.
17. **Wong D.** Salivary diagnostics powered by nanotechnologies, proteomics and genomics. *JADA*. 2006; 137: 313–21.
18. **Arunkumar JS, Burde KN, Shakunthala GK.** Developments in diagnostic applications of saliva in oral and systemic diseases - A comprehensive review. *J Sci Innov Res*. 2014; 3: 372–87.
19. **Wang TH, Peng YH, Zhang CY, *et al.*** Single-molecule tracing on a fluidic microchip for quantitative detection of low-abundance nucleic acids. *J Am Chem Soc*. 2005; 127: 5354–9.
20. **Wang J, Chen Z, Corstjens PL, *et al.*** A disposable microfluidic cassette for DNA amplification and detection. *Lab Chip*. 2006; 6: 46–53.
21. **Song L, Walt DR.** Fiber-optic microsphere-based arrays for multiplexed biological warfare agent detection. *Anal Chem*. 2006; 78: 1023–33.
22. **Yager P, Edwards T, Fu E, *et al.*** Microfluidic diagnostic technologies for global public health. *Nature*. 2006; 442: 412–8.
23. **Herr AE, Hatch AV, Throckmorton DJ, *et al.*** Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proc Natl Acad Sci USA*. 2007; 104: 5268–73.
24. **Robertson NM, Hizir MS, Balcioglu M, *et al.*** Discriminating a single nucleotide difference for enhanced miRNA detection using tunable graphene and oligonucleotide nanodevices. *Langmuir*. 2015; 31: 9943–52.
25. **Wang Z, Zhang J, Guo Y, *et al.*** A novel electrically magnetic-controllable electrochemical biosensor for the ultra sensitive and specific detection of attomolar level oral cancer-related microRNA. *Biosens Bioelectron*. 2013; 45: 108–13.
26. **Hizir MS, Balcioglu M, Rana M, *et al.*** Simultaneous detection of circulating oncomiRs from body fluids for prostate cancer staging using nanographene oxide. *ACS Appl Mater Interfaces*. 2014; 6: 14772–8.

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27. **Tan Y, Wei X, Zhao M, *et al.*** Ultraselective homogeneous electrochemical biosensor for DNA species related to oral cancer based on nicking endonuclease assisted target recycling amplification. *Anal Chem*. 2015; 87: 9204–8.
28. **Wei F, Yang J, Wong DT.** Detection of exosomal biomarker by electric field-induced release and measurement (EFIRM). *Biosens Bioelectron*. 2013; 44: 115–21.
29. **Kim J, Valdés-Ramírez G, Bandodkar AJ, *et al.*** Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites. *Analyst*. 2014; 139: 1632–6.
30. **Kim J, Imani S, de Araujo WR, *et al.*** Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. *Biosens Bioelectron*. 2015; 74: 1061–8.
31. **Zilberman Y, Chen Y, Sonkusale SR, *et al.*** Dissolved ammonia sensing in complex mixtures using metalloporphyrin-based optoelectronic sensor and spectroscopic detection. *Sens. Actuators B Chem*. 2014; 202: 976–83.
32. **Mannoor MS, Tao H, Clayton JD, *et al.*** Graphene-based wireless bacteria detection on tooth enamel. *Nat Commun*. 2012; 3: 763.
33. **Cottat M, D'Andrea C, Yasukuni R, *et al.*** High sensitivity, high selectivity SERS detection of MnSOD using optical nanoantennas functionalized with aptamers. *J Phys Chem C*. 2015; 119: 15532–40.
34. **Liang YH, Chang CC, Chen CC, *et al.*** Development of an Au/ZnO thin film surface plasmon resonance-based biosensor immunoassay for the detection of carbohydrate antigen 15-3 in human saliva. *Clin Biochem*. 2012; 45: 1689–93.
35. **Zhang Y, Chen R, Xu L, *et al.*** Silicon nanowire biosensor for highly sensitive and multiplexed detection of oral squamous cell carcinoma biomarkers in saliva. *Anal Sci*. 2015; 31: 73–8.
36. **Priyanka N, Kalra N, Shanbhag N, *et al.*** Recent approaches in saliva as a credible periodontal diagnostic and prognostic marker. *AOS*. 2012; 2: 40–6.
37. **Wei F, Wong DTW.** Point-of-care platforms for salivary diagnostics. *Chin J Dent Res*. 2012; 15: 7–15.
38. **Wei F, Patel P, Liao W, *et al.*** Electrochemical sensor for multiplex biomarkers detection. *Clin Cancer Res*. 2009a; 15: 4446–52.
39. **Latorre A, Posch C, Garcimartín Y, *et al.*** Single-point mutation detection in RNA extracts using gold nanoparticles modified with hydrophobic molecular beacon-like structures. *Chem Commun*. 2014; 50: 3018.
40. **Tu M, Chia D, Wei F, *et al.*** Liquid biopsy for detection of actionable oncogenic mutations in human cancers and electric field induced release and measurement liquid biopsy (eLB). *Analyst*. 2016; 141: 393–402.
41. **Valentini P, Fiammengo R, Sabella S, *et al.*** Gold-nanoparticle-based colorimetric discrimination of cancer-related point mutations with picomolar sensitivity. *ACS Nano*. 2013; 7: 5530–8.
42. **Zhang HD, Zhou J, Xu ZR, *et al.*** DNA mutation detection with chip-based temperature gradient capillary electrophoresis using a slantwise radiative heating system. *Lab Chip*. 2007; 7: 1162.
43. **Wang H, Liu W, Zhang X, *et al.*** Toward point-of-care testing for JAK2 V617F mutation on a microchip. *J Chromatogr A*. 2015; 1410: 28–34.
44. **Wang Z, Gerstein M, Snyder M.** RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 2009; 10: 57–63.
45. **Byron SA, Van Keuren-Jensen KR, Engelthaler DM, *et al.*** Translating RNA sequencing into clinical diagnostics: opportunities and challenges. *Nat Rev Genet*. 2016; 17: 257–71.
46. **Spielmann N, Ilsley D, Gu J, *et al.*** The human salivary RNA transcriptome revealed by massively parallel sequencing. *Clin Chem*. 2012; 58: 1314–21.
47. **Burgos KL, Javaherian A, Bomprezzi R, *et al.*** Identification of extracellular miRNA in human cerebrospinal fluid by next-generation sequencing. *RNA*. 2013; 19: 712–22.
48. **Hu L, Wu C, Guo C, *et al.*** Identification of microRNAs predominately derived from testis and epididymis in human seminal plasma. *Clin Biochem*. 2014; 47: 967–72.
49. **Bahn JH, Zhang Q, Li F, *et al.*** The landscape of microRNA, piwi-interacting RNA, and circular RNA in human saliva. *Clin Chem*. 2015; 61: 221–30.
50. **Quinn JF, Patel T, Wong D, *et al.*** Extracellular RNAs: development as biomarkers of human disease. *J Extracell Vesicles*. 2015; 4: 27495.
51. **Eswaran J, Horvath A, Godbole S, *et al.*** RNA sequencing of cancer reveals novel splicing alterations. *Sci Rep*. 2013; 3: 1689.
52. **Vardiman JW, Jüergen T, Arber DA, *et al.*** The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009; 114: 937–51.
53. **Gambino G, Tancredi M, Falaschi E, *et al.*** Characterization of three alternative transcripts of the BRCA1 gene in patients with breast cancer and a family history of breast and/or ovarian cancer who tested negative for pathogenic mutations. *Int J Mol Med*. 2015; 35: 950–6.
54. **Crowley E, Di Nicolantonio F, Loupakis F, *et al.*** Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol*. 2013; 10: 472–84.
55. **Diaz LA Jr, Bardelli A.** Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014; 32: 579–86.
56. **Lin CC, Huang WL, Wei F, *et al.*** Emerging platforms using liquid biopsy to detect EGFR mutations in lung cancer. *Expert Rev Mol Diagn*. 2015; 15: 1427–40.
57. **Alix-Panabières C, Pantel K.** Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov*. 2016; 6: 479–91.
58. **Tabernero J, Lenz HJ, Siena S, *et al.*** Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. *Lancet Oncol*. 2015; 16: 937–48.
59. **Spindler KLG, Pallisgaard N, Vogelius I, *et al.*** Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. *Clin Cancer Res*. 2012; 18: 1177–85.
60. **Freidin MB, Freydina DV, Leung M, *et al.*** Circulating tumor DNA outperforms circulating tumor cells for KRAS mutation detection in thoracic malignancies. *Clin Chem*. 2015; 61: 1299–304.
61. **Taly V, Pekin D, Benhaim L, *et al.*** Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. *Clin Chem*. 2013; 59: 1722–31.
62. **Nakauchi C, Kagara N, Shimazu K, *et al.*** Detection of TP53/PIK3CA mutations in cell-free plasma DNA from metastatic breast cancer patients using next generation sequencing. *Clin Breast Cancer*. 2016; pii: S1526-8209(16)30100-8: doi:10.1016/j.clbc.2016.05.004.
63. **Thierry AR, Mouliere F, El Messaoudi S, *et al.*** Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. *Nat Med*. 2014; 20: 430–5.
64. **Asano H, Toyooka S, Tokumo M, *et al.*** Detection of EGFR gene mutation in lung cancer by mutant-enriched polymerase chain reaction assay. *Clin Cancer Res*. 2006; 12: 43–8.
65. **Chen WW, Balaj L, Liau LM, *et al.*** BEAMing and droplet digital PCR analysis of mutant IDH1 mRNA in glioma patient serum and cerebrospinal fluid extracellular vesicles. *Mol Ther Acids*. 2013; 2: e109.
66. **Diehl F, Li M, Dressman D, *et al.*** Detection and quantification of mutations in the

plasma of patients with colorectal tumors. *Proc Natl Acad Sci USA*. 2005; 102: 16368–73.

67. **Kim HR, Lee SY, Hyun DS, *et al.*** Detection of EGFR mutations in circulating free DNA by PNA-mediated PCR clamping. *J Exp Clin Cancer Res*. 2013; 32: 50.
68. **Zimmerman JW, Jimenez H, Pennison MJ, *et al.*** Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. *Chin J Cancer*. 2013; 32: 573–81.
69. **Huang X, El-Sayed MA.** Gold nanoparticles: optical properties and implementations in cancer diagnosis and photothermal therapy. *J Adv Res*. 2010; 1: 13–28.
70. **Cormio L, Vedruccio C, Giorgio L, *et al.*** Non-invasive electromagnetic detection of bladder cancer. *ISRN Urol*. 2014; 802328: 1–4.
71. **Pu D, Liang H, Wei F, *et al.*** Evaluation of a novel saliva-based epidermal growth factor receptor mutation detection for lung cancer: a pilot study. *Thorac Cancer*. 2016; 7: 428–36.
72. **Yang J, Wei F, Schafer C, *et al.*** Detection of tumor cell-specific mRNA and protein in exosome-like microvesicles from blood and saliva. *PLoS ONE*. 2014; 9: e110641.
73. **Brain JD, Kavet R, McCormick DL, *et al.*** Childhood leukemia: electric and magnetic fields as possible risk factors. *Environ Health Perspect*. 2003; 111: 962–70.
74. **Johansen C, Nielsen OR, Olsen JH, *et al.*** Risk for leukemia and brain and breast cancer among Danish utility workers: a second follow-up. *Occup Environ Med*. 2007; 64: 782–4.
75. **Klaeboe L, Blaasaas KG, Haldorsen T, *et al.*** Residential and occupational exposure to 50-Hz magnetic fields and brain tumors in Norway: a population-based study. *Int J Cancer*. 2005; 115: 137–41.
76. **Erren TC.** Biologically based study of magnetic field exposure and female breast cancer—will there be a sensible interpretation without information on a likely culprit? *Epidemiology*. 2003; 14: 129–30.
77. **Kliukiene J, Tynes T, Andersen A.** Residential and occupational exposures to 50-Hz magnetic fields and breast cancer in women: a population-based study. *Am J Epidemiol*. 2004; 159: 852–61.
78. **Panagopoulos DJ, Johansson O, Carlo GL.** Polarization: a key difference between man-made and natural electromagnetic fields, in regard to biological activity. *Sci Rep*. 2015; 5: 14914.
79. **Lewczuk B, Redlarski G, Żak A, *et al.*** Influence of electric, magnetic, and electromagnetic fields on the circadian system: current stage of knowledge. *Biomed Res Int*. 2014; 2014: 169459.
80. **Choi YE, Kwak JW, Park JW.** Nanotechnology for early cancer detection. *Sensors (Basel)*. 2010; 10: 428–55.
81. **Mani V, Chikkaveeraiah B, Patel V, *et al.*** Ultrasensitive immunosensor for cancer biomarker proteins using gold nanoparticle film electrodes and multienzyme-particle amplification. *ACS Nano*. 2009; 3: 585–94.
82. **Lässer C, Eldh M, Lötvall J.** Isolation and characterization of RNA-containing exosomes. *J Vis Exp*. 2012; 59: 3037.
83. **Wei F, Liao W, Xu Z, *et al.*** A bio-abiotic interface constructed by nanoscale DNA-dendrimer and conducting polymer for ultrasensitive bio-molecular diagnosis. *Small*. 2009b; 5: 1784–91.
84. **Wei F, Chien-Chung LAJ, Ziding F, *et al.*** Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer. *Am J Respir Crit Care Med*. 2014; 190: 1117–26.