Microchemical Journal 168 (2021) 106506

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Saliva Lab-on-a-chip biosensors: Recent novel ideas and applications in disease detection

Melika Haji Mohammadi [a], Sterre Mulder [b], Patricia Khashayar [c], Alireza Kalbasi [d], Mostafa Azimzadeh [e,f,g,*], Amir Reza Aref [h,*]

[a] Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
[b] Leiden University Medical Center, Leiden University, Leiden, the Netherlands
[c] Center for Microsystems Technology, Imec and Ghent University, Ghent, Zwijnaarde, Belgium
[d] Brigham and Women's Hospital, Harvard Medical University, Boston, MA, USA
[e] Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[f] Medical Nanotechnology & Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, 89195-999 Yazd, Iran
[g] Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, 89195-999 Yazd, Iran
[h] Belfer Center for Applied Cancer Science, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Keywords:
Metabolic
Saliva
lab-on-a-chip (LOC)
Biosensor
Disease

ABSTRACT

Lab-on-a-chip (LOC) devices have revolutionized the metabolic detection field by bringing together the advantages of biosensors and microfluidics in a single chip. Saliva, which contains a variety of biomarkers, has recently been recognized as a non-invasive biofluidic alternative for many diagnostic purposes. Hence, the application of LOCs for saliva analysis can be a promising diagnostic technique for a wide range of medical situations and diseases. Herein we present a review on the LOCs applied so far for diseases detection using saliva and future horizons. In this regard, after a brief introduction of LOC and saliva, a list of novel LOCs designed or used for the detection of different bacteria and viruses, cytokines, metabolites, kidney related diseases, prostate cancer and oral diseases along with their pros and cons is provided. We are also going to report how these advancements could help with the current pandemic.

1. Introduction

In this article, we review recent advances in the application of lab-on-a-chip (LOC) biosensors for the detection of different diseases using saliva samples. Several biosensors and/or biochip devices in the form of LOC devices have been developed to detect biomarkers linked to a variety of diseases and health situations using saliva samples. To our knowledge, only a few reviews in the field of LOCs have assessed the application of saliva as the target; they are, however, not comprehensive nor updated. Moreover, the existing reviews are either limited to biosensors for oral and periodontal diseases [1], wearables [2–5], or those detecting cancer [6]. As a result, in this review, we have tried to illustrate an overview of recent advancements in the field of saliva lab-on-a-chips for both oral and systematic diseases along with their pros and cons, and what to expect in the future.

First, a brief introduction to lab-on-a-chip devices and their applications in medicine and metabolic biomarker assays is given. Then, the importance of using saliva as a biomarker source is discussed. Finally, the advancements in saliva biomarker analysis using lab-on-a-chip devices in the recent five years will be explained.

1.1. Lab-on-a-chip (LOC) and their applications in metabolite detection

According to the MeSH (Medical Subject Headings) definition, LOC devices are "Microdevices that combine microfluidics technology with electrical and/or mechanical functions for analyzing very small fluid volumes" (https://www.nlm.nih.gov/mesh/meshhome.html). The integration of biosensors into the microfluidic chips has revolutionized clinical diagnosis over the past two decades [7,8]. These LOCs have been used for the detection/quantification of a variety of cells, biomarkers,

* Corresponding authors at: Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran (M. Azimzadeh).
E-mail addresses: m.azimzadeh@ssu.ac.ir (M. Azimzadeh), Amir_Aref@hms.harvard.edu (A.R. Aref).

https://doi.org/10.1016/j.microc.2021.106506
Received 5 April 2021; Received in revised form 1 June 2021; Accepted 3 June 2021
Available online 6 June 2021

pathogens, and contaminants in a vast number of papers and commercial products [9,10]. In fact, these simple and portable devices resemble miniaturized laboratories as they can perform several laboratory tasks including sample manipulation and biomarker detection anywhere, using small amounts of the sample [11]. This quality enables us to perform a variety of sample manipulation and pre-treatments, including but not limited to lysis, cleaning, extraction, isolation, separation, preconcentration, mixing, and amplification of the metabolites using the same device [12–14]. This along with the fact that LOCs use small amounts of the sample can help improve the sensitivity and selectivity of these methods compared with conventional metabilte analysis methods such as mass spectrometry (MS), nuclear magnetic resonance (NMR), and chromatography methods [15,16].

The low quantity of target metabolites and the presence of several potential interference molecules are the main limitations of using metabolites as biomarkers; LOCs, however, can solve this problem [10,17]. In addition, they can help overcome other limitations of the conventional metabolic analysis techniques such as high cost, being time-consuming, high complexity, and need of expensive devices and expert personnel. LOCs can even perform metabolite analysis of single-cell that requires precise handling and high accuracy [18,19].

A variety of target specimens (blood, saliva, sweat, tear, etc.), sample handling steps and techniques, detection methods (existing transducers are electrochemical, colorimetric, fluorescent, etc.), and applications have been listed for LOCs [8,14,20–23]. To name a few examples, LOC have been used for metabolites like cell-free DNAs and RNAs [24], exosomes and other extracellular vesicles [25,26], glucose and albumin [27,28], lactate [29]; oxygen, carbon dioxide, and pH [30], reactive oxygen species [10], and growth factors and protiens [31]. The recent COVID-19 pandemic taught us the urgent need of having a simple, sensitive, and fast detection technique for rapid and early diagnosis to save more lives. By the time this article is published, many biosensors and microfluidics have been developed to detect COVID-19 through different platforms and methods, some of which are mentioned in this article [32,33].

In addition, real-time and portable LOC devices can act as a point-of-care (POC) device for the detection of several metabolites for a variety of disease [34]. When coupled with smartphone for analysis and/or reporting the results, they are considered as a good choice for screening purposes, especially in-home testing or in remote areas. On the other hand, novel fabrication methods such as 3D printing and innovative sample handling and analysis methods, including artificial intelligence (A.I.) and Machine Learning, have turned LOCs into an attractive option for for scientists and companies [35–38].

1.2. Saliva as a source of metabolic biomarkers

In modern healthcare systems, the application of less invasive sampling techniques is one of the main priorities; thus, more recent LOC devices are racing towards the use of easy and non-invasive sampling sources. Saliva is an ideal specimen in this regard since it represents the physiological and pathological state of the body, even in the case of tumors that are known to be highly heterogonous [39–41]. This is mainly because numerous substances are diffused from the blood into the saliva, which mostly consists of water (99%) [42,43]. There are reports suggesting that saliva can contain DNA, RNA, exosomes, minerals, hormones, mucins, enzymes, expectorated secretions from bronchus and nasal cavity, serum and blood derivatives from oral wounds, microorganisms, and their products, and last but not least, food debris [44–46].

Moreover, saliva sampling is more convenient for patients than collecting blood or tears, and no additional action is needed to prevent clotting as in blood samples. Furthermore, the lower risk of personnel contamination while using saliva is another justification to move from blood to saliva [47,48]. Despite all this, its potential to be used as a liquid biopsy to detect and monitor different biomarkers has not been understood until recently [49–51].

2. Saliva LOCs to detect biomarkers for various diseases

Here we discuss recent advancements in LOCs for saliva assessments by categorizing them based on the disease type.

2.1. Bacteria and virus detection

The ability to detect bacteria or viruses rapidly is a key step towards more effective and accurate treatments in the clinical practice; this, however, remains a challenge both in developed and developing countries, especially in cases where subjects have low or undetectable levels of the pathogen [52]. Most of the traditional detection methods are culture-based, whereas newer techniques apply PCR analyses. These methods both require special sample preparation steps and thus are time-consuming and expensive [53 54]. As a result, low cost and portable microfluidic devices are currently the focus of many studies, which their recent advances are summarized in Table 1.

2.1.1. Helicobacter pylori (H. Pylori)

One of the earliest saliva-based wearable platforms for bacterial detection was introduced by Mannoor et al. in 2012 [55]. Initially, they printed graphene on water-soluble silk, which was later bio-transferred onto bovine tooth enamel. The detection of specific bacteria even at a single-cell level was achieved by self-assembly of antimicrobial peptides on the graphene surface (Fig. 1A). Considering its resonant circuit-based nature, the device worked properly without on-board power, and was suitable for wireless monitoring. As a test, the authors decided to detect H. pylori, one of the major pathogens involved in the development of duodenal ulcers. They demonstrated that the device was able to detect as low as 100 cells in 1 μL of the sample (and up to 106 cells), which is below the minimum infectious dose for this bacterium. They claimed that the battery-free highly sensitive graphene nanosensor integrated in tooth enamel as a biomaterial could enable remote monitoring of pathogenic bacteria and used for various purposes. However, being semi-selective, its application was limited [55].

2.1.2. Bacillus cereus (B. Cereus)

In 2007, Chen et al. introduced a LOC for detecting infectious agents particularly B. cereus [56]. The device was capable of processing intact cells in saliva at a concentration of 106 cells/mL and prepared them for a PCR-based analysis. After this initial success, the authors envisaged the use of the device for the detection of viral or other bacterial pathogens. In 2010, some researchers designed a microfluidic cassette for detecting pathogens in saliva, based on the presence of nucleic acids [57]. The main advantage of the chip was that all reagents were pre-coated on the device. The cell lysis, nucleic acid isolation, amplification and detection steps all happened on the chip. The user, thus, only needed to introduce the unprocessed sample to the system. The cassette was validated for the detection of B. cereus and viral armored RNA of HIV and HIV I. The detection limit for bacteria detection was estimated to range from 103 to 104 cells/mL; whereas that for virion detection was in the order of 105 virions/mL. The authors anticipated that the system could be used for the detection of other pathogens after adjusting primers and other reagents (Fig. 1B).

2.1.3. Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) is a globally spread virus that can cause acquired immunodeficiency syndrome (AIDS). Due to its ease of transmission, early diagnosis is key to limit its spread [61]. It has been proven that the highest viral titers are detected at the early stages of infection [66]; therefore, RNA amplification might represent an effective tool for early diagnosis of the virus.

In 2016, Chen et al. created a microfluidic chemical and reagent device (CARD) to detect anti-HIV antibodies and viral RNA

Table 1
Recently developed biochips/biosensors for pathogen detection with saliva as sample (NM = not mentioned).

Disease/Application	Target(s)	Recognition Element Type	Limit of Detection (LoD)	Operation Time	Ref.
B. cereus	Nucleic acid	DNA/RNA biosensor	10^6 cells/mL	10 min	[56]
B. cereus and HIV	Nucleic acid and HIV viral RNA	DNA/RNA biosensor	10^3-10^4 bacteria/mL and 10^5 virions/mL	10 min	[57]
COVID 19	SARS-CoV-2 RNA	RNA biosensor	75 copies of virus RNA	30 min	[58]
COVID 19	COVID Spike Protein	LFA Immunosensor	NM	less than10 min	[59]
COVID 19	COVID Spike Protein	LFA Immunosensor	NM	NM	[60]
H. pylori	*H. pylori*	Immunosensor	100 cells/μL	15 min	[55]
HIV	HIV viral RNA and antibodies	DNA/RNA biosensor and Immunosensor	NM	80 min	[61]
P. aeruginosa	Pyocyanin	Immunosensor	0.5 μM	NM	[62]
S. aureus	MSSA and MRSA genomic DNA	DNA/RNA biosensor	30 fg (8–10 copies)	2.5 h	[63]
Zika virus	Zika viral RNA	DNA/RNA biosensor	857 RNA copies/mL	3–10 min	[64]
Zika virus	anti-ΔNS1 and anti-EDIII	Immunosensor	17 fg/mL and 53 fg/ml respectively	NM	[65]

simultaneously [61]. This was a newer generation of that developed in 2010 for the same application [57]. The new device aimed at early HIV infection diagnosis, even when antibodies were not yet detectable. The automated system carried out the required steps, after loading the sample and reagents. Isolation of viral RNA was performed through magnetic bead-based purification followed by reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) assay, which is roughly three-fold faster than the conventional RT-PCR methods. Moreover, the presence of anti-HIV antibodies was analyzed by an immunoassay using Lateral flow assay (LFA) with HIV viral glycoproteins. Finally, both biomarkers were detected using fluorescence. The tool had several advantages, making it especially attractive for low-resource settings: the ability of simultaneous detection of the antibody and HIV-RNA that helped with screening for and controlling the HIV epidemic; it did not require trained personnel and worked on blood samples as well as saliva, the latter was non-invasive and thus easier to collect.

2.1.4. *Pseudomonas aeruginosa (P. aeruginosa)*

P. aeruginosa represents a substantial challenge in clinic due to the innate ability to cause antibiotic resistance resulting in impenetrable biofilms. Thus, its rapid detection is pivotal for choosing the appropriate antibiotic treatment and preventing possible complications [67]. When host defense mechanism is compromised, the patients are more susceptible to P. aeruginosa linked with respiratory tract infections [68]. These bacteria produce, amongst others, the toxic metabolite pyocyanin (PYO). PYO is known to be specific to P. Aeruginosa, which is a potential biomarker enabling its detection [62].

In 2017, Žukovskaja et al. tested a droplet-based microfluidic chip that worked based on the surface-enhanced Raman spectroscopy (SERS) technique [62] (Fig. 1C). It measured enhanced Raman signal from aggregated silver nanoparticles. The signal was induced by PYO after mixing the biological sample with a silver colloidal solution and certain salts. Then, a laser beam was focused on the sample and the back-scattered light was detected. The chip was able to detect PYO in real saliva samples with a detection limit of 0.5 μM. Considering the clinically relevant concentrations of PYO, which range between 7 and 130 μM, this sensor was reported to be sensitive enough for clinical application. However, the reading of concentrations above 55 μM were less accurate, due to surface saturation at this threshold. One of the promising features of this chip was that the same LOC-SERS technique could be used to detect other bacteria metabolites.

2.1.5. *Staphylococcus aureus (S. aureus)*

Oblath et al. designed a microfluidic chip for the detection of S. aureus in spiked saliva, based on DNA identification [63]. Pre-warmed samples (up to 95 °C), which consisted of lysed cells, were added to the chip and the genomic DNA was extracted by filtration through a nanoporous aluminum oxide membrane. The presence of bacteria was then confirmed by the identification of both methicillin-susceptible (MSSA) and -resistant (MRSA) S. aureus DNA using RT-PCR. Streptococcus mutants were used as control organisms. The chip was able to detect as low as 30 fg (8–10 copies) of MSSA DNA after amplification to 300 fg (100–125 copies). Because the designed chip had seven separated wells, the device was capable of identifying different bacteria simultaneously after choosing appropriate primers and probes. Such a chip would be useful in distinguishing certain infections with overlapping symptoms. However, as for the majority of oral infections and linked diseases such as pancreatic cancer, the concentration of the organism/biomarker is more important rather than studying only its presence; as a result, a quantitative real-time test is required.

2.1.6. *Zika virus*

Certain infectious diseases spread globally and therefore have been responsible for several outbreaks during the last years [64]. Early detection of these contagious infections is key to prevent their further spread. Zika virus, for example, has been linked with adverse pregnancy complications such as microcephaly and other neurological damages in the unborn infants. Diagnosis of Zika virus infection is difficult since its vectors, symptoms and geographical distribution are comparable to those of Dengue virus and other Flaviviruses [69]. It could be detected using PCR- and enzyme-linked immunosorbent assay (ELISA)-based methods. While the former is too complex to be implemented in rural areas, the latter is not sensitive enough [70]. Moreover, the virus can only be detected in blood 1–7 days before the clinical manifestations. In saliva, urine and semen, however, it is detectable for a longer time. Thus, saliva is believed as the preferred biofluid for these tests because of its non-invasive ease of collection, cost effectiveness and high acceptance by subjects.

In 2018, Sabalza et al. transformed a microfluidic device developed to detect HIV viral RNA into a tool to detect viral RNA of Zika virus in saliva samples without the need of prior RNA purification [64]. In order to do so, they used an RT-LAMP assay coupled to reverse dot-blot technique (RDB). After heat-induced lysis, the samples were subjected to isothermal amplification by a RT-LAMP assay using three primer pairs that targeted the conserved regions of the capsid gene of the Zika virus. The amplified sequences were RDB hybridized to membrane-bound capture probes, which were then detected by streptavidin-conjugated HRP. Using human saliva artificially spiked with Zika virus, the researchers showed the limit of detection (LoD) of the tool to be 8.57x102 viral copies/mL. The test took 3–10 min, depending on the concentration of the virus in the saliva. This platform represented a model, which could be easily modified and used for detecting other emerging pathogens such as malaria [71].

2.1.7. *Influenza virus*

Bhardwaj et al. detected influenza H1N1 virus using a combination of electrochemical and colorimetric measurements [72] (Fig. 1D). Their sample pad contained two papers with pore sizes of 11 and 0.45 μm in diameter. This led to moderate and uniform flow. This helped concentrate the horseradish peroxidase-tagged antibody-H1N1 complexes, providing the opportunity to read low concentrations of the virus.

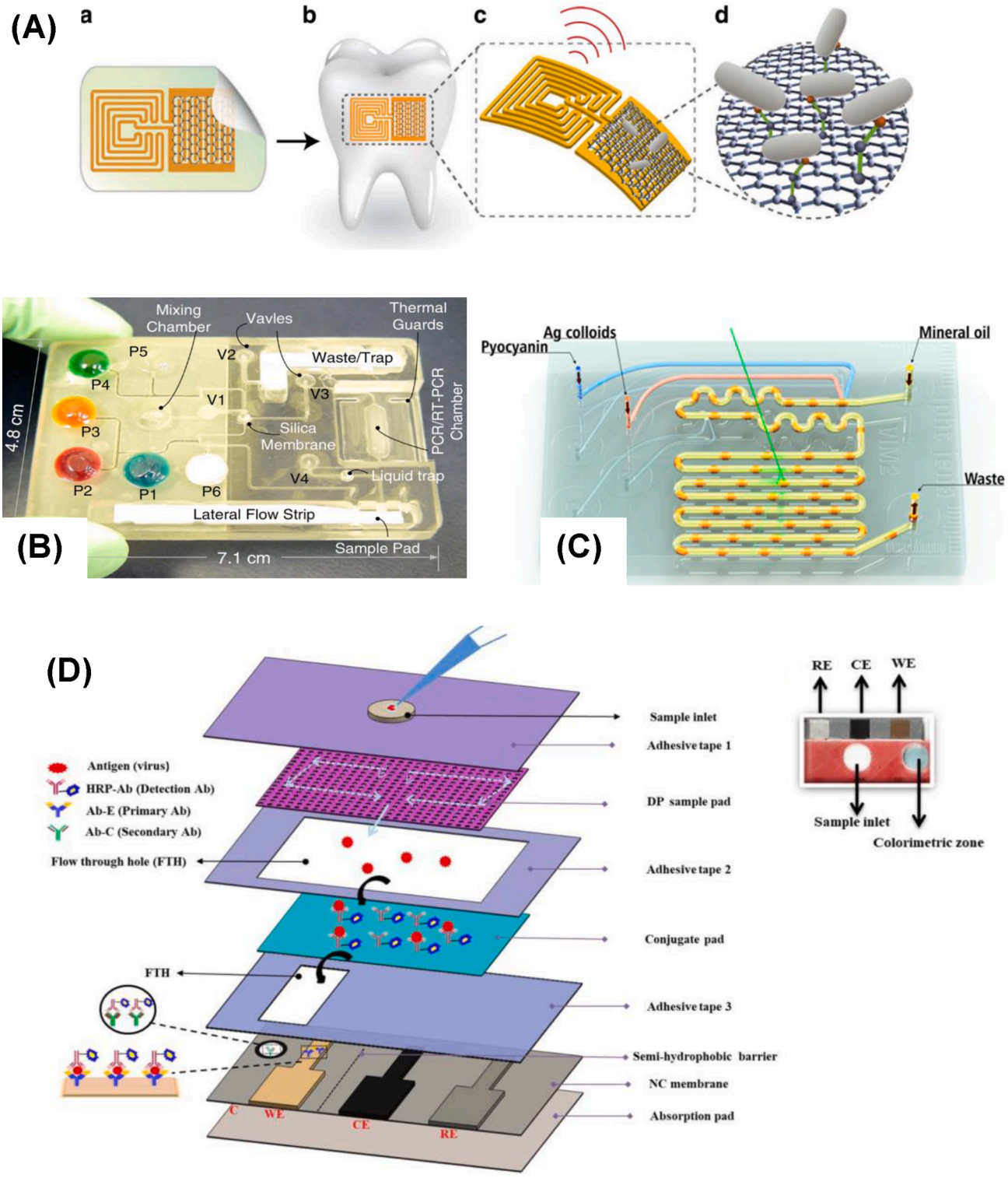

Fig. 1. (A) Detection of H1N1 virus in saliva, a) The graphene was printed on silk; b) then transferred onto the tooth surface; c) A magnified image of the biosensor with wireless readout; d) illustration of pathogen binding to the graphene by self-assembled peptides [55]; (B) Microfluidic cassette designed by Chen et al. for the detection of pathogens B. cereus [57]; (C) Droplet-based microfluidic chip that uses SERS method to assess the presence of pyocyanin (PYO), specific to P. aeroginosa [62] (D) (left) Schematic representation, and the (right) photograph of the dual biosensor [72]. Reprinted with permissions.

Moreover, it guaranteed that only small sized bioparticles such as virus could pass through, improving the specificity. The system thus worked based on colometric and electrochemical impedance spectroscopy (EIS) measurements simultaneously, which helped reduce the risk of false positive. The LoD for the sensor was 4.7 plaque forming units (PFU)/mL by EIS and 2.27 PFU/mL by colometric with a turnaround time of 6 min. The authors found the reproducibility, stability and selectivity of the device to be sufficient for the clinical application. They also proposed the sample pad to be used for virus detection in air samples, since large particles could be filtered out.

2.1.8. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): COVID 19

The rapid increase in the confirmed cases of SARS-CoV-2 infection, commonly known as COVID-19, has turned the infection into an uncontrollable pandemic. So far, according to the WHO official counts, this outbreak has resulted in over 108,000,000 confirmed cases of COVID-19, including over 2,300,000 deaths, roughly (https://covid19.who.int/) [73]. The diagnosis of this single-stranded positive-sense RNA virus from the coronavirus (CoVs) clade is mainly based on the detection of viral RNA through real-time reverse transcription PCR on respiratory specimens. This is while some recent studies have shown that saliva can replace nasopharyngeal swabs, reducing the patients' discomfort as well as need for specialized medical personnel and viral transmission risk to the operator [32,74]. Using this biofluid to the microfluidic PoC has also resulted in reduced diagnosis time, which is critical in the current pandemic.

In an attempt, Linnes et al adopted their microRapid Autonomous Analytical Device (microRAAD) platform to detect SARS-CoV-2 RNA. This platform is based on reverse-transcription loop mediated isothermal amplification (RT-LAMP) and can detect as few as 75 copies of the virus RNA in saliva samples within 30 min [58]. In order to increase the turnaround time and increase the testing yield, many researchers have looked into immunosensors. In this regard, a Rapid Salivary Test (RST) based on the LFA was developed by Azzi et al to detect SARS-CoV-2. The antibody-based test detected the presence of the spike protein in a salivary sample in less than 10 min, detecting. The appearance of two colored bands (both test and control lines) suggests that the subject is infected with a high sensitivity (93%) [59]. In another attempt, Ymbern et al developed a capillary-driven microfluidic technology for rapid and quantitative covid-19 serological testing in saliva. Domino Capillaric Circuits (DCC) is an autonomous lateral flow assay developed through 3D printing [60].

2.2. Cytokines

Cytokines are small proteins released by cells and are known to influence on the intracellular interactions [75]. They are associated with several diseases and can therefore be used for their diagnosis at early stages. Hao et al. developed a portable nanosensing system that detected cytokine concentrations using aptamers [76]. In the absence of cytokines, the aptamers are flexible and unfolded, whereas their formation stabilizes once bounded with a cytokine. This brings the negatively charged cytokines closer to the graphene surface, resulting on changes in surface charge distribution. These changes are measured as the current of free electron carriers. The system used graphene-based field effect transistor (GFET), which enabled the wireless transmission of the results to a smartphone or cloud server and enabling the online visualization of the changes in the cytokine concentrations by the doctors. The portable nanosensing system had a LoD of 12 pM for IL-6 with a turnaround time of about 400 s.

2.3. Hormones and metabolites

2.3.1. Cortisol monitoring

It has been proven that stress, which is an inseparable part of nowadays' life, could act as a driver for certain diseases, including cardiovascular disorders [77] and diabetes [78]. Currently, its diagnosis is often based on self-questionnaires and interviews, which are subjective and can introduce bias. As a more objective approach, cortisol has been shown to serve as a biomarker to indicate both psychological stress and certain diseases such as Cushing's syndrome and Addison's disease [79]. The steroid hormone released from the adrenal cortex plays an important role in the regulation of blood pressure, sleep/wake cycle, carbohydrate and glucose metabolism among others. Like other biomarkers, the conventional measurement methods for cortisol measurement are usually limited to central laboratories, due to the need for complex equipment or labelling preparation processes, which are both expensive and time-consuming [80]. Moreover, the existing techniques provide a snapshot of the subject's cortisol level, while real-time and continuous monitoring of cortisol levels is required to assist doctors in better diagnosis and treatment of cortisol-related conditions. However, some promising techniques have been recently developed (Table 2).

In 2017, Pan et al. proposed a paper-based biochip that was able to detect cortisol concentrations using an electrochemical immunosensing techniques [81]. Anti-cortisol antibodies were conjugated to gold microelectrodes using a monolayer of 3,3′-Dithiodipropionic acid di(N-hydroxysuccinimide) ester. In this method, the increase in the electrical resistance of the gold electrode caused by the binding of cortisol to the antibodies was measured. The tool had a LoD of 3 pg/mL. The detection process took 12 min and its result was comparable with that of ELISA. The high sensitivity of the sensor and the possibility of continuous measurement and transferring data through wide-area networks for real-time monitoring were among its main advantages.

A line-free (free from power supply lines) microfluidic mass sensor was created by Yamaguchi et al. in 2018 [82]. It consisted of a mechanical resonator, an element for the excitement of vibrations and a microfluidic mechanism for washing out the impurities from the sample. The sample was immobilized on the resonator, the frequency of which was proportional to the mass of anti-cortisol antibodies binding to the cortisol in the sample. As a result, the measured frequencies were translated into cortisol levels present in saliva samples. After analysis, a dissociation solution is added to cleave cortisol from antibodies so that they could be used for the next test. The whole cycle took about 50 min, which is considerably faster than the conventional ELISA method (about 4 h). The LoD was found to be 0.879 ng/mL, which is sufficient for cortisol monitoring. For the above outlined reasons, this line-free sensor is mainly advantageous for low resource areas.

Khan et al. developed an electrochemical immunosensor based on EIS measurements for cortisol concentration evaluation in saliva in 2019 [83]. To improve the accuracy of the device, they optimized both the saliva incubation time and the concentration of covalently attached anti-cortisol antibodies on the surface of the electrode as well as using bovine serum albumin as sensitivity enhancer. The optimized chip was able to detect as low as 0.8 pg/ml of cortisol, without reporting any interference with ascorbic acid. The validation tests showed good correlation with ELISA measurements. However, the device should be tested using more saliva samples with different physiological condition before being used in clinical practice.

A new polymer LOC based on microfluidic capillary flow assay was developed by Vinitha et al. to determine the concentrations of unbound cortisol in saliva [84]. The portable fluorescent analyser offered quantitative measurement of cortisol with high accuracy. All the steps such as the incubation of sample with the antibodies and the positive/negative controls were integrated on the disposable chip (Fig. 2A). The detection was based on the measurement of fluorescent emission from the antibodies coupled with the dried fluorescent dye binding to cortisol molecules captured on the surface. The linear range of the tool using spiked artificial saliva was 7 pg/ml-16 ng/ml. This non-invasive PoC is especially interesting for stress analysis and could be an ideal too for low-resource settings after being validated with real saliva samples. Using smartphone based analysis can make the test cheaper and more practical.

In 2020, Ducker et al developed iPro Cube, a portable lateral flow device (LFD) for cortisol and alpha-amylase measurement in saliva [85]. The tool showed reliable results in measuring the markers at-rest and after intense exercise, with a moderate-to-large correlation with ELISA results (0.53–0.81). The limit of quantification (LOQ) of the tool was 0.58 nmol/L for cortisol, making it an interesting tool for those interested in studying psychophysiological stress.

2.3.2. Glucose monitoring

Effective self-management in diabetic patients is often challenging as

Table 2
Recently developed biochips/biosensors for cortisol monitoring with saliva as target sample (NM = not mentioned).

Disease/Case of Use	Target(s)	Recognition Element Type	Limit of Detection (LoD)	Time of operation	Ref.
Stress and related diseases	Cortisol	Immunosensor	3 pg/mL	12 min	[81]
Stress and related diseases	Cortisol	Immunosensor	0.879 ng/mL	50 min	[82]
Stress and related diseases	Cortisol	Immunosensor	0.8 pg/mL	12 min	[83]
Stress and related diseases	Cortisol	Immunosensor	NM	10 min	[86]
Psychophysiological stress	Cortisol	Lateral flow	0.58 nmol/L	NM	[85]

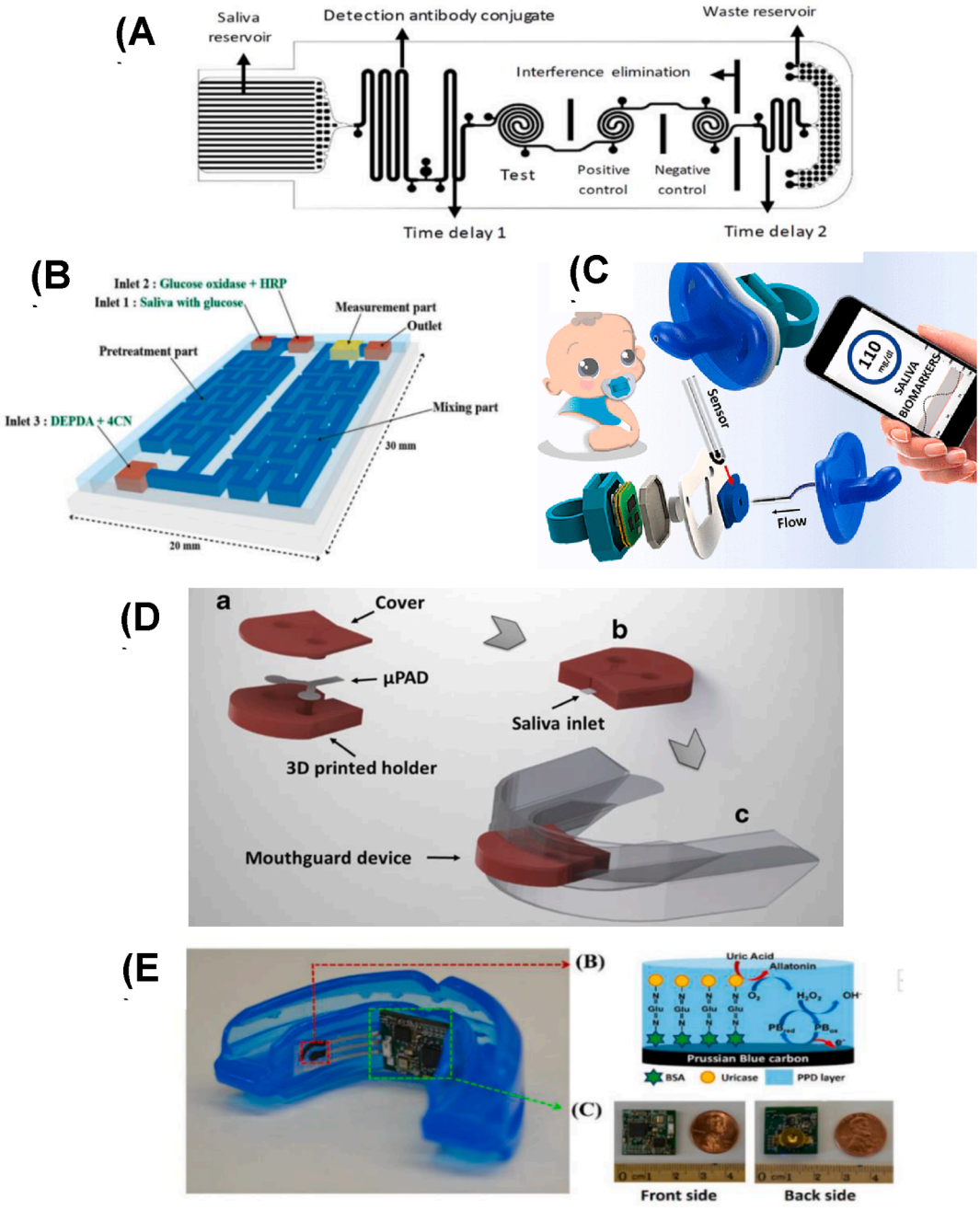

Fig. 2. (A) Structure of the designed chip for cortisol measurement in saliva contains capturing and detection of cortisol with positive and negative control zones [84]; (B) Platform designed by Jung et al. for glucose measurement in saliva via micro-electro-mechanical system and optical measurement technology [88]; (C) schematic representation of the designed pacifier glucose biosensor works by mobile phone [50]; (D) Assembling a 3D-printed holder into a wearable paper-based devices for salivary glucose monitoring [91]; (E) Fabricated mouthguard for the continuous measurement of uric acid in the saliva [101]. Reprinted with permissions.

it requires regular monitoring of blood glucose levels. Having a non-invasive method to achieve this goal, thus, is appreciated. Many studies have suggested saliva as a preferable non-invasive alternative to determine glucose levels [87]. Saliva-based measurement techniques, however, needs to be more sensitive as glucose concentrations are several hundred-folds lower in saliva compared with blood [88]. Several

attempts have been made to produce microfluidic devices that can replace blood-based methods for glucose quantification (Table 3).

From the earliest attempts in this field, the microfluidic platform developed by Srinivasan et al. in 2003 [89] is worth mentioning. They generated a platform that was compatible with different body fluids including serum, plasma, urine and saliva as the target sample. The platform could measure the glucose content based on the Trinder's reaction, which is a colorimetric enzyme-kinetic assay. The system also showed promising results measuring other metabolites such as lactate, glutamate and pyruvate.

In 2017, Jung et al. manufactured a portable LOC glucose sensor that could determine as low as a few milligrams of glucose in saliva within 4 min [88] (Fig. 2B). The injection of saliva and glucose oxidase into separate inlets in the device resulted in the oxidation of glucose and formation of H2O2. Subsequently, a coloring mixture of horseradish peroxidase (HRP), N,N'-diethyl-p-phenylenediamine and 4-chloro-1-naphtol was injected and mixed with the saliva. HRP converted H2O2 into water and oxygen, making the saliva blue. Using a light-emitting diode and a photodiode, the absorbance of the saliva was measured at 630 nm. The authors demonstrated that the output current corresponded with the concentration of glucose in the saliva sample. The short detection time and low LoD were the main advantages of this sensor.

To monitor glucose levels in infants, Carmona et al. developed a portable saliva-based sensor for continuous glucose monitoring [50]. A nontoxic polymeric nipple to be suckled by the infants provided a unidirectional flow for saliva collection, making the tool more practical for infants compared with the existing invasive methods or wearable devices. The glucose-oxidase enzyme was immobilized on the electrode using chitosan; the oxidization of glucose resulted in detectable changes in the current that was later on read by a Prussian Blue electrode transducer (Fig. 2C). The functionality of this sensor was tested in type I diabetic patients. The results in saliva were comparable to that in blood, showing the accuracy of the test. After improving the system's safety for long-term use, this biosensor could be used to detect other relevant biomarkers in order to monitor the infant's health as well as early diagnosis of metabolic conditions.

A paper-based colorimetric biosensor was developed by Jia et al. for a similar purpose [90]. The device benefited from the nanomaterial properties to enhance the performance of the assay. The problem linked with the heterogeneity of color distribution - a major drawback in most paper-based microfluidic devices - was solved by coating the paper-based microfluidic analytical device (μPAD) with graphene oxide. The graphene oxide catalyzed the enzymatic reaction that helped with the measurement. Due to the photolithography technique used in the fabrication of the device, a detection limit of about 0.02 mM and a linear dynamic range of 0–1 mM was achieved. Later on, the authors developed a smartphone application for colorimetric detection of the target using a MATLAB-based image quantitative analysis. In this way, the device became portable and easy to use. Despite the higher LoD of the device compared with its counterparts, being portable made it more acceptable among the users [68].

In 2019, Castro et al. produced a μPAD integrated into a silicone mouthguard as the first paper-based microfluidic portable sensor for the measurement of glucose [91]. A 3D-printed holder, in which the μPAD was inserted, proved to be an essential piece to successfully integrate the sensor in the mouthguard (Fig. 2D). It prevented any contact between the mouth and the reagents, lowering possible risks. Glucose concentration was measured by inducing a reaction between glucose, HRP and GOx. The resulted solution was then colored with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonic acid, and the colorimetric detection was performed at 510 nm wavelength. Using this technique, glucose was measured accurately with a LoD of 27 μmol/L and in a linear range of 0–2 mmol/L, which is considerably below the normal concentration in healthy people (0.04 to 0.34 mmol/L) [92]. Since diabetic patients tend to have higher glucose concentrations, it is expected that the sensor will determine the glucose concentration accurately in real saliva samples. The sensor was also able to measure the concentration of nitrite, which will be discussed later in more detail. Being wearable, provided the patients with the opportunity to conveniently perform self-monitoring. The cheap, simple, fast and biodegradable chip seemed an attractive alternative to conventional blood-based methods.

2.4. Kidney related diseases detection

2.4.1. Chronic kidney disease

Chronic kidney disease defines a large group of disorders characterized by altered kidney structure function. It is often the result of underlying conditions such as diabetes and hypertension, and can lead to metabolic abnormalities, cardiovascular disease, cognitive dysfunction and even death [95]. Early diagnosis is pivotal to prevent disease progression and blood urea nitrogen (BUN) and urea are the most common surrogates used to assess the kidney function [96]. Many studies have shown a good correlation between blood and salivary urea levels [97,98].

In 2019, Wang et al. reported an inexpensive real-time sensing system to determine urea concentrations in saliva samples [96]. It consisted of a tank linked to two optical fibers. Input light signal was delivered by the fiber connected to a LED, while sensing signal was detected by the other optical fiber linked to a CdS photo-conductive cell. Only 1 μL of the sample was needed to perform the test. In this regard, the sample was mixed with a urease and pH indicator solution. Urease hydrolyzed urea into carbon dioxide and ammonia, which, in combination with water, formed ammonium and hydroxyl ions. These reactions increased the pH in the solution, resulting in a change in the current of the photo-conductive cell. These changes were correlated with the urea concentration. The dynamic range of the tool was 24–300 mg/dL, showing a good correlation between salivary urea and blood BUN. In other words, the system allowed easy and rapid testing of urea concentrations (detection time less than 20 s), which is of great importance in patients with chronic kidney disease.

2.4.2. Hyperuricemia

Hyperuricemia (i.e. high levels of uric acid) can be caused by either reduced excretion or increased intake of uric acid [99]. This condition may result in gout and kidney stones or increase the risk of developing type 2 diabetes [100]. Both diagnosis of the disease and the assessment of the treatment efficacy require uric acid monitoring. In 2015, Kim et al. developed a mouthguard capable of continuous real time uric acid measurements [101]. This device contained an uricase-modified electrode system and miniaturized electronics, including a Bluetooth low

Table 3
Recently developed biochips/biosensors for glucose monitoring with saliva as target sample (NM = not mentioned).

Disease/Case of Use	Target(s)	Recognition Element Type	Limit of Detection (LoD)	Time of operation	Ref.
Glucose monitoring	Glucose	Enzyme-based	0.02 mM	NM	[90]
Glucose monitoring	Glucose	Enzyme-based	10–100 mg/dL	4 min	[88]
Glucose monitoring	Glucose	Enzyme-based	27 μmol/L	NM	[91]
Glucose monitoring	Glucose	Enzyme-based	9–100 mg/dL	NM	[89]
Glucose monitoring	Glucose	Enzyme-based	0.1 mM	Continuous	[93]
Glucose monitoring	Glucose	Enzyme-based	0.02 mM	NM	[94]

energy transceiver. Uricase, immobilized on screen-printed electrodes using bovine serum albumin and glutaraldehyde as crosslinker, catalyzed the reaction with uric acid to produce allantoin and hydrogen peroxide (Fig. 2E). The latter was then reduced by hydrogen peroxidase, generating a current proportional to the uric acid concentration in the saliva sample. The results were similar to that of tests performed using a lab-scale potentiostat (R2 = 0.998). The low energy usage of the Bluetooth transceiver made the wearable biosensor capable of running for 12 h to 5 days, depending on the frequency of measurements. In short, the mouthguard with integrated electrochemical biosensor chip was proved practical, highly sensitive and able to continuous monitoring of uric acid levels in the normal range (100–250 μM) and up to 600 μM. The biosensor was reported to be modifiable to measure other biomarkers.

2.4.3. Kidney failure

Hemodialysis is frequently used in patients with kidney diseases to help filter out waste products from blood. Unfortunately, there is no simple way to effectively monitor the progression of hemodialysis, although some biomarkers such as nitrite, urea or potassium have been suggested for this purpose [102]. Nitrite is diffused from blood into saliva and has been shown to be a potential biomarker for hemodialysis monitoring while using saliva samples [103].

In 2010, Klasner et al. designed a paper-based microfluidic device for nitrite quantification in artificial saliva as well as glucose and ketone measurement in urine [102]. Nitrite levels were measured using the Griess reaction, based upon a colorimetric assay. As the device was designed for hemodialysis monitoring, relative quantification of nitrite before and after the procedure was considered more important than measuring the exact concentration of nitrite at a certain time point. The authors succeeded in detecting nitrite levels as low as 5 μM and up to 2000 μM, a suitable range for clinical detection assays. They used a new polymer that permitted the fabrication of smaller channel features and thus helped with the complexity of the device.

Table 4 illustrates a list of other biosensors developed to measure biomarkers for kidney or prostate-related diseases in saliva.

2.5. Prostate cancer detection

Prostate cancer is the third most common malignancy among men and its early detection is crucial to avoid metastasis and poor outcomes [105]. Prostate specific antigen (PSA) is a potential biomarker for both early diagnosis and determination of therapeutic response [106]. Currently, PSA levels are measured using serum-based techniques that require automated analyzers in laboratories, which is both time-consuming and costly [104].

In 2019, Khan et al. proposed a paper-based graphene-polymer-Au biosensor for the detection of PSA in saliva samples [104]. The base layer was the composite of graphene nanoplatelets and amphiphilic polymer (PS67-b-PAA27) spin-coated on a Whatman filter paper. The anti-PSA antibodies were conjugated on Au electrodes using dithiobis (succinyl propionate). Upon binding of PSA to the antibodies, the resistance of the graphene-polymer coating increased linearly depending on its concentration. The detection range of the sensor was 100 fg/mL to 100 ng/mL, with a LoD as low as 40 fg/mL. This is a broader measurement range compared with other existing/reported methods. Thus, the biosensor provided a fast, sensitive and highly selective way of testing PSA in saliva samples, showing a 94% agreement with ELISA. The biosensor had a shelf-life of about 7 weeks.

Table 4
Recently developed biochips/biosensors for kidney and prostate related diseases with saliva as target sample (NM = not mentioned).

Disease/Case of Use	Target (s)	Recognition Element Type	Limit of Detection (LoD)	Time of operation	Ref.
Chronic kidney disease	Urea	Enzyme-based	25.5 mg/dL	20 sec	[96]
Hemodialysis monitoring	Nitrite	Enzyme-based	5 μM	25 min	[102]
Hyperuricemia	Uric acid	Enzyme-based	NM	Continuous	[101]
Prostate cancer detection	PSA	Immunosensor	40 fg/mL	3–5 min	[104]

2.6. Special cases of oral diseases

2.6.1. Multi-species biofilm modeling and drug evaluation

In 2013, Nance et al. produced a high-throughput microfluidic chip for developing an in vitro multi-species oral biofilm to evaluate the effectiveness of cetylpyridinium chloride as an antimicrobial agent [107] (Fig. 3A). The most important advantage of such a device was that it mimicked the in vivo community of species, rendering more reliable results for clinical practice. The generated biofilm included species such as Aggregatibacter, Fusobacterium, Neisseria, Porphyromonas, Streptococcus and Veillonella, which are usually found in a typical human dental plaque.

One year later, Samarian et al. exploited the same chip as a high-throughput in-vitro system to generate 3D biofilm reconstructions for further subculture and DNA extraction and analysis [108]. This system used natural saliva as a nutrient source, which is desirable to mimic in-vivo communities in research studies. The same concept can be utilized for developing other multi-species communities using urine or wound exudates; the drawback of such method is that it may not be suitable for long-term studies.

2.6.2. Oral cancer

Squamous cell carcinoma is the most common type of oral cancer, which affects different parts of the oral cavity such as soft palate, tongue and gingiva. Common signs include ulcers with induration or mucosal nodularity. In a small number of patients, pain and bleeding may also be present [109]. Despite recent advancements in medical technology, the 5-year survival rate of these patients remains quite low. This is mainly due to late diagnosis, given that the majority of patients are diagnosed when they have reached stage III or IV of the disease [110]. At these stages, not only medical treatment becomes less effective, but also surgery becomes riskier to perform [111].

These patients are generally diagnosed based on interview questions during history taking that include patients' family history and lifestyle, along with observations of oral fibrosis or white plaques, and biopsy analysis. These methods are ineffective, especially in cases without noticeable signs at early stages [112]. Many attempts have been therefore made to find suitable biomarkers enabling early detection of oral cancer.

In 2018, Lin et al. introduced a microfluidic chip for the detection of anti-P53 autoantibody as a biomarker for early detection of oral cancer [111]. Instead of forcing the fluid to move towards different reaction wells, they used magnetic beads to carry the biomarker along the designed path. In brief, in the first well, the antigen-coated beads interacted with the sample, which contained the corresponding antibodies. After washing, the beads entered and bounded to the well coated with HRP-conjugated secondary antibodies. After the addition of the substrate, the optical density of the reaction was measured and the concentration of the antibodies of interest were determined (Fig. 3C). The authors reported that the detection limit of the chip was 4 ng/mL, which is lower than that of the conventional plate-based ELISA. Furthermore, the magnetic beads not only increased the surface area but also helped reduce the operational time by 3-fold to approximately 1 h. Despite its promising results, its use in clinical practice was limited to cancers in which the anti-P53 autoantibodies were present.

In 2019, Dong et al. manufactured an optical microfluidic biosensor for simultaneous detection of several protein biomarkers in spiked saliva

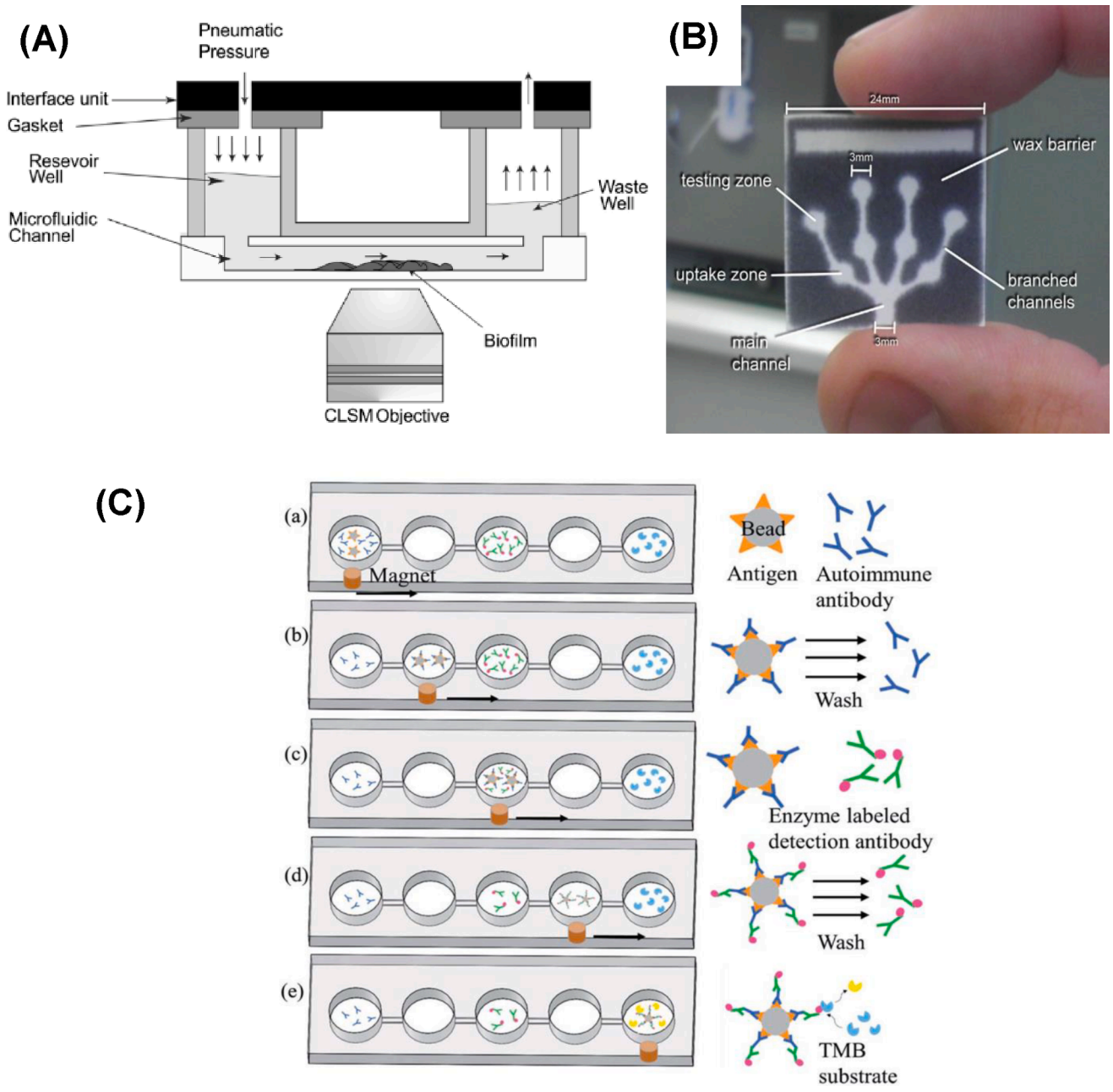

Fig. 3. (A) Microfluidic chip designed for developing a multi-species oral biofilm in a single channel [107]; (B) Redesigned μPAD manufactured by Bhakta et al. for nitrite detection in the branched channels. [119]; (C) Workflow of a device engineered for the detection of anti-P53 autoantibody from saliva samples using Magnetic beads with p53 antigen and optical readout. Reprinted with permission [111]. Reprinted with permissions.

[113]. The detection process was based on the absorbance measurements using polyethylenimine-modified polythiophene-C70, a sensitive organic photodetector, which was aligned to the antibody-coated chambers. The validation tests showed good correlation with ELISA; although the reported higher readings was possibly due to the differences between the viscosity of artificial saliva used for calibration and real saliva. The authors also demonstrated that the LoD for IL-8, IL-1β and MMP-8 were between 80 pg/mL and 120 pg/mL. From among them, the LoD of IL-8 was considerably below the clinical threshold (600 pg/mL), making the device suitable for early diagnosis of oral cancer. Being low-cost and portable turned the presented device into an attractive POC not only for oral cancer diagnosis but also for other diseases.

2.6.3. *Periodontitis*

Periodontitis is a multifactorial gum infection, caused secondary to complex interactions between periodontal bacteria, host immune system and other risk factors. The most common form is plaque-induced with pocket formation. It manifests with swollen and bleeding gingiva, causing the formation of periodontal lesions in the long run. It can also result in tooth loss unless treated. Periodontitis is also shown to be linked with other diseases such as acute coronary syndrome and systemic sclerosis [114,115]. While its progression is site-specific, clinical differentiation of the affected site is difficult. Early diagnosis of the disease is also impossible, as the current diagnostic and screening tools including clinical and radiographical examinations suffer from lack of sensitivity and specificity. Recent studies, thus, have focused on finding and validation of specific biomarkers in saliva that are linked with the disease [116].

Wignarajah et al. manufactured a colorimetric biosensor for the detection of two inflammatory biomarkers, namely Human Neutrophil

Elastase and Catepsin-G, linked with periodontitis [117]. The designed multiplex biosensor composed of protease specific magnetic beads covalently bounded to the gold sensor surface. The detection process started with the cleavage of magnetic beads with proteases, resulting in a golden color visible to the naked eye. It was rapid with no wash, labeling or amplification step, making it suitable for unskilled personnel. The detection limit for Human Neutrophil Elastase and Catepsin-G was 1 pg/mL and 0.1 pg/mL, respectively. This biosensor displayed higher sensitivity and specificity compared to previously developed diagnostic tools.

Nitrite represents another potential biomarker for the diagnosis and subsequent monitoring of periodontitis. One of the common tools used to quantify nitrite is based on a spectrophotometric method, mostly based on the Griess reaction [118]. The main disadvantage of the method, however, is the need for large volumes of sample and reagent. In order to overcome the need for a spectrophotometer and making the device more accessible, in recent years, researchers have focused on the design of POC devices for nitrite quantification.

In 2014, Bhakta et al. developed a μPAD by wax printing for nitrite quantification in saliva [119]. Under optimized conditions, the modified Griess reaction-based device was capable of determining nitrite in the 10–1000 μM range with a detection limit of 10 μM.

Recently, the previously discussed μPAD produced by Castro et al. for glucose detection [91]. The tool was able to measure nitrite concentration as low as 7 μmol/L and up to 400 μmol/L in saliva. Nitrite was detected by performing a modified Griess reaction using citric acid, sulfanilamide solution and N-(1-naphthyl)ethylenediamine solutions and followed by colorimetric analysis. The μPAD was manufactured using a craft cutter printer, which not only simplified the production process but also reduced its price considerably [119].The low-cost and biodegradable nitrite (and glucose) monitoring platform had slightly lower LoD compared with similar devices and was capable of continuous detection (Fig. 3B).

The prognostic relevance of matrix metalloproteinase (MMP), particularly MMP-8 and MMP-9, for periodontitis has been proven in several studies [120]. The first commercially available POC immunoassay for the detection of MMP-8 was launched under PerioSafe [121]. The LFA not only could be used for diagnosis or monitoring of periodontitis, but also was capable of identifying adults genetically predisposed to develop the disease [122]. The test results were available in 5 min and did not require trained personnel. The sensitivity and specificity of the device were 76.5% and 96.7% respectively. The value of 25 ng of MMP8 per milliliter of filtrate, derived from 5 mL mouth rinse, was determined as the cutoff for elevated risk of periodontitis [123].

Recently, Lee et al. determined human odontogenic ameloblast-associated (ODAM) protein in gingival crevicular fluid as a representative biomarker for periodontitis [124]. ELISA has long been the most common method to measure ODAM concentrations. However, it is not suitable to be used as a POC diagnostic, which is the main clinical application of such devices. As a result, Lee et al. developed an aptasensor in 2019 [125]. They screened different aptamers using methods such as circular dichroism and surface plasmon resonance before selecting a pair. The LoD for these two aptamers used in a sandwich-type SPR biosensor were 0.24 nM and 1.63 nM. The next simplified generation, which was a sandwich-type LFA, was still capable of detecting ODAM protein as low as 8.32 nM and 14.59 nM in buffer and spiked-saliva, respectively. In addition, they confirmed its specificity using other non-target molecules and normal saliva. Its high sensitivity for ODAM proteins made the aptasensor a good alternative for periodontitis monitoring.

2.6.4. *Peri-implantitis*

Peri-implantitis is an inflammation of *peri*-implant tissue that is more aggressive than periodontitis, suggesting its prevention or early detection even more critical. So far, the progression of the disease has been assessed with radiographs, which, is of little help in early diagnosis [116].

It has been demonstrated that MMP-8 in oral fluids not only is a potential predictive biomarker for the diagnosis of *peri*-implantitis [126] but also its increased level is suggestive of disease progression [127]. ImplantSafe is a rapid lateral flow immunosensor that can be used for implant checkups and early diagnosis of *peri*-implantitis. It can also help assess the efficiency of treatment for mucositis and *peri*-implantitis. The sample, which is *peri*-implant sulcus fluid, can be collected using the provided strip. While a negative result, (appearance of the control line alone) suggests a negligible risk of *peri*-implantitis. A positive result (appearance of both control and sample line), however, is not straight forward and other factors such as age of the implant and radiographical assessments should be taken into consideration before making the final diagnosis [116]. Despite all this, the technique could be a useful addition to conventional radiographs in the assessment of *peri*-implantitis. Table 5 illustrates a list of published biosensors or biochips for saliva analysis to detect oral diseases.

3. Conclusion

Here we explained how the non-invasive detection of different diseases using saliva could improve clinical management of the patients, providing them with opportunity of early diagnosis and more efficient treatment monitoring. This is mainly because saliva contains a variety of biomarkers linked with the detection of both oral and non-oral diseases. This article explains the application of such tools in identifying bacteria and viruses, monitoring glucose and cortisol levels, detection of kidney disease, prostate cancer, oral cancers and other oral related conditions among many others. We have also explained in details the strategies use for the manufacturing of these devices, their advantages and sensitivity along with their shortcomings.

Such tools allow an easy, non-invasive and rather painless sample collection. These platforms are also portable and thus could be used anywhere, even by unskilled personnel. Their generally low cost and rapid turnaround time are among other benefits of such devices. The latter is of great importance especially because some of these sensors are designed for pandemics, where accessibility, low cost and being user friendly plays a key role. Moreover, the ease of sample collection represents a major advantage for the detection of target biomarkers without interference from other non-specific biomolecules.

The main challenges of such devices are linked to the complexity of manufacturing, their working mechanisms and the difficulty in commercialization. Another challenge relates to the complex properties of saliva, its high viscosity and, for some biomarkers, low or hardly detectable concentrations. This complicates detecting a complete range of biomarkers for diagnostics and monitoring purposes. Overall, the ease and non-invasiveness of the procedure outweigh the above-mentioned problems.

4. Future perspectives

In brief, saliva LOC biosensors provide an encouraging horizon for revolutionizing patient management, while making it more accessible for everyone. This need becomes more prominent nowadays when the world is struggling with the outbreak of COVID-19, and rapid, accurate and sensitive detection techniques with non-invasive sample collection that would allow early diagnosis and intervention are needed more than ever.

In other words, lab-on-a-chip biosensors, especially those based on non-invasive liquid biopsy with no need for needle and/or operation, are considered the future of medical diagnosis. In this regard, saliva seems like be one of the most promising sample sources as it contains many biomarkers proved to be useful for diagnosis, screening and prognosis of not only oral diseases but also many other systematic disorders. The list of these diseases is not limited to what mentioned in this review as everyday scientists discover new biomarkers in saliva linked with

Table 5
Recently developed biochips/biosensors for oral diseases with saliva as target sample (NM = not mentioned).

Disease/Case of Use	Target(s)	Recognition Element Type	Limit of Detection (LoD)	Time of operation	Ref.
Oral cancer	Anti-P53 autoantibody	Immunosensor	4 ng/mL	60 min	[111]
Oral cancer	IL-8, IL-1β and MMP-8	Immunosensor	90, 80 and 120 pg/mL respectively	30 min	[113]
Peri-implantitis	aMMP-8	Immunosensor	NM	NM	[116]
Periodontitis	Human Neutrophil Elastase and Catepsin-G	Enzyme-based	1 pg/mL and 0.1 pg/mL respectively	NM	[117]
Periodontitis	Nitrite	Enzyme-based	10 μM	NM	[119]
Periodontitis	aMMP-8	Immunosensor	25 ng/mL	5–10 min	[121]
Periodontitis	Nitrite	Enzyme-based	7 μmol/L	Continuous	[91]
Periodontitis	ODAM protein	Immunosensor	14.59 nM	NM	[125]

certain disorders. Therefore, combining the advantages of biosensor-integrated lab-on-a-chips and saliva can be considered as a futuristic medical diagnostic approach. The widespread application of this concept has already been used in the current COVID pandemic and has saved many lives.

Home tests, on the other hand, are becoming more popular as telemedicine and virtual clinics tries to overcome geographical barriers and increase access to healthcare services. Telemedicine refers to the practicing medicine remotely as the healthcare provider and patient are not physically present in one place. Remote Patient Monitoring is one of the main aspects of telemedicine and allows remote caregivers to monitor the patients at home by using mobile medical devices to collect data. Easy to use home tests with non-invasive sample collection are thus a prerequisite for such technologies and thus saliva lab-on-chips can be of great interest in the evolution of telemedicine. Wearable chips/biosensors using saliva that can transmit data to the smartphones and internet of things are the main evolutions making this possible.

Having said all this, we expect future researches to be more focused on improving the sensitivity and specificity of such tools, aiming to improve their performance and accuracy. In addition, considering the application, safe and biocompatible materials are needed for those sensors aimed to be implanted or incorporated in wearables for real time continuous monitoring of the underlying condition. Innovative biocomposites could be one of the options in this regard. Finally yet importantly, use of artificial intelligence and machine learning is growing fast in medicine and biology. This could also change the future of the LoCs as it can evolve the monitoring process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] L. Steigmann, S. Maekawa, C. Sima, S. Travan, C.-W. Wang, W.V. Giannobile, Biosensor and Lab-on-a-chip Biomarker-identifying Technologies for Oral and Periodontal Diseases, Front. Pharmacol. 11 (2020) 1663.
[2] J. Kim, A.S. Campbell, B.-E.-F. de Ávila, J. Wang, Wearable biosensors for healthcare monitoring, Nat. Biotechnol. 37 (2019) 389–406.
[3] F. Wen, T. He, H. Liu, H.-Y. Chen, T. Zhang, C. Lee, Advances in chemical sensing technology for enabling the next-generation self-sustainable integrated wearable system in the IoT era, Nano Energy 105155 (2020).
[4] V. Mani, T. Beduk, W. Khushaim, A.E. Ceylan, S. Timur, O.S. Wolfbeis, K. N. Salama, Electrochemical sensors targeting salivary biomarkers: A comprehensive review, TrAC, Trends Anal. Chem. 116164 (2020).
[5] F. Yi-Qiang, G. Feng, W. Mei, J. Zhuang, T. Gang, Y.-J. Zhang, Recent development of wearable microfluidics applied in body fluid testing and drug delivery, Chin. J. Anal. Chem. 45 (2017) 455–463.
[6] A. Tadimety, A. Closson, C. Li, S. Yi, T. Shen, J.X. Zhang, Advances in liquid biopsy on-chip for cancer management: Technologies, biomarkers, and clinical analysis, Crit. Rev. Clin. Lab. Sci. 55 (2018) 140–162.
[7] T. Leichle, L. Nicu, T. Alava, MEMS biosensors and COVID-19: missed opportunity, ACS Sensors (2020).
[8] L. Xu, N. Shoaie, F. Jahanpeyma, J. Zhao, M. Azimzadeh, K.T. Al-Jamal, Optical, electrochemical and electrical (nano)biosensors for detection of exosomes: A comprehensive overview, Biosens. Bioelectron. 161 (2020).
[9] Y. Xing, L. Zhao, Z. Cheng, C. Lv, F. Yu, F. Yu, Microfluidics-based sensing of biospecies, ACS Appl. Bio Mater. (2020).
[10] L. Lin, J.-M. Lin, Development of cell metabolite analysis on microfluidic platform, J. Pharm. Anal. 5 (2015) 337–347.
[11] E.K. Sackmann, A.L. Fulton, D.J. Beebe, The present and future role of microfluidics in biomedical research, Nature 507 (2014) 181–189.
[12] K. Kant, M.-A. Shahbazi, V.P. Dave, T.A. Ngo, V.A. Chidambara, L.Q. Than, D. D. Bang, A. Wolff, Microfluidic devices for sample preparation and rapid detection of foodborne pathogens, Biotechnol. Adv. 36 (2018) 1003–1024.
[13] M.S. Chiriacò, M. Bianco, A. Nigro, E. Primiceri, F. Ferrara, A. Romano, A. Quattrini, R. Furlan, V. Arima, G. Maruccio, Lab-on-chip for exosomes and microvesicles detection and characterization, Sensors 18 (2018) 3175.
[14] K.M. Koo, E.J. Wee, Y. Wang, M. Trau, Enabling miniaturised personalised diagnostics: from lab-on-a-chip to lab-in-a-drop, Lab Chip 17 (2017) 3200–3220.
[15] L.-Y. Hung, C.-H. Wang, C.-Y. Fu, P. Gopinathan, G.-B. Lee, Microfluidics in the selection of affinity reagents for the detection of cancer: paving a way towards future diagnostics, Lab Chip 16 (2016) 2759–2774.
[16] J.R. Kraly, R.E. Holcomb, Q. Guan, C.S. Henry, Review: Microfluidic applications in metabolomics and metabolic profiling, Anal. Chim. Acta 653 (2009) 23–35.
[17] F.S. Iliescu, D.P. Poenar, F. Yu, M. Ni, K.H. Chan, I. Cima, H.K. Taylor, I. Cima, C. Iliescu, Recent advances in microfluidic methods in cancer liquid biopsy, Biomicrofluidics 13 (2019), 041503.
[18] D. Feng, T. Xu, H. Li, X. Shi, G. Xu, Single-cell Metabolomics Analysis by Microfluidics and Mass Spectrometry: Recent New Advances, J. Anal. Test. 4 (2020) 198–209.
[19] K. Matuła, F. Rivello, W.T.S. Huck, Single-cell analysis using droplet microfluidics, Adv. Biosyst. 4 (2020) 1900188.
[20] S.N. Topkaya, M. Azimzadeh, M. Ozsoz, Electrochemical biosensors for cancer biomarkers detection: recent advances and challenges, Electroanalysis 28 (2016) 1402–1419.
[21] E.E. Augspurger, M. Rana, M.V. Yigit, Chemical and biological sensing using hybridization chain reaction, ACS Sensors 3 (2018) 878–902.
[22] A. Nilghaz, L. Guan, W. Tan, W. Shen, Advances of paper-based microfluidics for diagnostics—the original motivation and current status, ACS Sensors 1 (2016) 1382–1393.
[23] S.M. Seifati, N. Nasirizadeh, M. Azimzadeh, Nano-biosensor based on reduced graphene oxide and gold nanoparticles, for detection of phenylketonuria-associated DNA mutation, IET Nanobiotechnol. 12 (2017) 417–422.
[24] C.D.M. Campos, S.S.T. Gamage, J.M. Jackson, M.A. Witek, D.S. Park, M. C. Murphy, A.K. Godwin, S.A. Soper, Microfluidic-based solid phase extraction of cell free DNA, Lab Chip 18 (2018) 3459–3470.
[25] Q. Zhu, M. Heon, Z. Zhao, M. He, Microfluidic engineering of exosomes: editing cellular messages for precision therapeutics, Lab Chip 18 (2018) 1690–1703.
[26] P. Zhang, J. Crow, D. Lella, X. Zhou, G. Samuel, A.K. Godwin, Y. Zeng, Ultrasensitive quantification of tumor mRNAs in extracellular vesicles with an integrated microfluidic digital analysis chip, Lab Chip 18 (2018) 3790–3801.
[27] N. Raj, V. Breedveld, D.W. Hess, Semi-enclosed microfluidic device on glass-fiber membrane with enhanced signal quality for colorimetric analyte detection in whole blood, Microfluid. Nanofluid. 25 (2021) 47.
[28] E.L. Rossini, M.I. Milani, L.S. Lima, H.R. Pezza, Paper microfluidic device using carbon dots to detect glucose and lactate in saliva samples, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 248 (2021), 119285.
[29] A. Chmayssem, N. Verplanck, C.E. Tanase, G. Costa, K. Monsalve-Grijalba, S. Amigues, M. Alias, M. Gougis, V. Mourier, S. Vignoud, A.M. Ghaemmaghami, P. Mailley, Development of a multiparametric (bio)sensing platform for continuous monitoring of stress metabolites, Talanta 229 (2021), 122275.
[30] I. Podunavac, S. Hinić, S. Kojić, N. Jelenčiakova, V. Radonić, B. Petrović, G. Stojanović, Microfluidic approach for measurements of pH, O2, and CO2in Saliva, Sens. Mater. 33 (2021) 1037–1050.
[31] I.F. Pinto, R.R.G. Soares, M.E.L. Mäkinen, V. Chotteau, A. Russom, Multiplexed microfluidic cartridge for at-line protein monitoring in mammalian cell culture processes for biopharmaceutical production, ACS Sensors 6 (2021) 842–851.
[32] A. Hosseini, R. Pandey, E. Osman, A. Victorious, F. Li, T. Didar, L. Soleymani, Roadmap to the Bioanalytical Testing of COVID-19: From Sample Collection to Disease Surveillance, ACS Sensors 5 (2020) 3328–3345.
[33] T. Leichle, L. Nicu, T. Alava, MEMS biosensors and COVID-19: missed opportunity, ACS Sensors 5 (2020) 3297–3305.
[34] W. Jung, J. Han, J.-W. Choi, C.H. Ahn, Point-of-care testing (POCT) diagnostic systems using microfluidic lab-on-a-chip technologies, Microelectron. Eng. 132 (2015) 46–57.
[35] J.B. Nielsen, R.L. Hanson, H.M. Almughamsi, C. Pang, T.R. Fish, A.T. Woolley, Microfluidics: Innovations in Materials and Their Fabrication and Functionalization, Anal. Chem. 92 (2020) 150–168.

[36] A. Lambert, S. Valiulis, Q. Cheng, Advances in optical sensing and bioanalysis enabled by 3D printing, ACS Sensors 3 (2018) 2475–2491.
[37] F. Cui, Y. Yue, Y. Zhang, Z. Zhang, H.S. Zhou, Advancing biosensors with machine learning, ACS Sensors 5 (2020) 3346–3364.
[38] V. Mehta, S.N. Rath, 3D printed microfluidic devices: a review focused on four fundamental manufacturing approaches and implications on the field of healthcare, Bio-Des. Manuf. 4 (2021) 311–343.
[39] E. Crowley, F. Di Nicolantonio, F. Loupakis, A. Bardelli, Liquid biopsy: monitoring cancer-genetics in the blood, Nat. Rev. Clin. Oncol. 10 (2013) 472.
[40] F.G. Bellagambi, T. Lomonaco, P. Salvo, F. Vivaldi, M. Hangouët, S. Ghimenti, D. Biagini, F. Di Francesco, R. Fuoco, A. Errachid, Saliva sampling: Methods and devices. An overview, TrAC, Trends Anal. Chem. 124 (2020), 115781.
[41] S. Dalirirad, D. Han, A.J. Steckl, Aptamer-Based Lateral Flow Biosensor for Rapid Detection of Salivary Cortisol, ACS Omega 5 (2020) 32890–32898.
[42] A.J. Bandodkar, J. Wang, Non-invasive wearable electrochemical sensors: a review, Trends Biotechnol. 32 (2014) 363–371.
[43] G. Iorgulescu, Saliva between normal and pathological. Important factors in determining systemic and oral health, J. Med. Life. 2 (2009) 303.
[44] M. Greabu, M. Battino, M. Mohora, A. Totan, A. Didilescu, T. Spinu, C. Totan, D. Miricescu, R. Radulescu, Saliva–a diagnostic window to the body, both in health and in disease, J. Med. Life. 2 (2009) 124–132.
[45] Y. Han, L. Jia, Y. Zheng, W. Li, Salivary exosomes: emerging roles in systemic disease, Int. J. Biol. Sci. 14 (2018) 633.
[46] J. Jiang, N.J. Park, S. Hu, D.T. Wong, A universal pre-analytic solution for concurrent stabilization of salivary proteins, RNA and DNA at ambient temperature, Arch. Oral Biol. 54 (2009) 268–273.
[47] K.E. Kaczor-Urbanowicz, C. Martin Carreras-Presas, K. Aro, M. Tu, F. Garcia-Godoy, D.T. Wong, Saliva diagnostics–Current views and directions, Exp. Biol. Med. 242 (2017) 459–472.
[48] M. Tiwari, Science behind human saliva, J. Nat. Sci. Biol. Med. 2 (2011) 53.
[49] A. Ilea, V. Andrei, C.N. Feurdean, A.-M. Băbțan, N.B. Petrescu, R.S. Câmpian, A. B. Boșca, B. Ciui, M. Tertiș, R. Săndulescu, Saliva, a magic biofluid available for multilevel assessment and a mirror of general health—a systematic review, Biosensors 9 (2019) 27.
[50] L. García-Carmona, A. Martín, J.R. Sempionatto, J.R. Moreto, M.C. González, J. Wang, A. Escarpa, Pacifier biosensor: toward noninvasive saliva biomarker monitoring, Anal. Chem. 91 (2019) 13883–13891.
[51] C.-Z. Zhang, X.-Q. Cheng, J.-Y. Li, P. Zhang, P. Yi, X. Xu, X.-D. Zhou, Saliva in the diagnosis of diseases, International, J. Oral Sci. 8 (2016) 133–137.
[52] U. Schaad, Which number of infecting bacteria is of clinical relevance? Infection 11 (1983) S87–S89.
[53] J. Mairhofer, K. Roppert, P. Ertl, Microfluidic systems for pathogen sensing: a review, Sensors 9 (2009) 4804–4823.
[54] P. Belgrader, W. Benett, D. Hadley, J. Richards, P. Stratton, R. Mariella, F. Milanovich, PCR detection of bacteria in seven minutes, Science 284 (1999) 449–450.
[55] M.S. Mannoor, H. Tao, J.D. Clayton, A. Sengupta, D.L. Kaplan, R.R. Naik, N. Verma, F.G. Omenetto, M.C. McAlpine, Graphene-based wireless bacteria detection on tooth enamel, Nat. Commun. 3 (2012) 763.
[56] Z. Chen, M.G. Mauk, J. Wang, W.R. Abrams, P.L. Corstjens, R.S. Niedbala, D. Malamud, H.H. Bau, A microfluidic system for saliva-based detection of infectious diseases, Ann. N. Y. Acad. Sci. 1098 (2007) 429–436.
[57] D. Chen, M. Mauk, X. Qiu, C. Liu, J. Kim, S. Ramprasad, S. Ongagna, W. R. Abrams, D. Malamud, P.L. Corstjens, An integrated, self-contained microfluidic cassette for isolation, amplification, and detection of nucleic acids, Biomed. Microdevices 12 (2010) 705–719.
[58] J.C. Linnes, N. Sritong, A. Colbert, K.F.K. Ejendal, Micro-rapid autonomous analytical device for sars-cov-2 detection, MicroTAS 2020 - 24th International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2020, pp. 1222-1223.
[59] L. Azzi, A. Baj, T. Alberio, M. Lualdi, G. Veronesi, G. Carcano, W. Ageno, C. Gambarini, L. Maffioli, S.D. Saverio, D.D. Gasperina, A.P. Genoni, E. Premi, S. Donati, C. Azzolini, A.M. Grandi, F. Dentali, F. Tangianu, F. Sessa, V. Maurino, L. Tettamanti, C. Siracusa, A. Vigezzi, E. Monti, V. Iori, D. Iovino, G. Ietto, A.d.S. L.R.S.T.N.s.R. Group, P.A. Grossi, A. Tagliabue, M. Fasano, Rapid Salivary Test suitable for a mass screening program to detect SARS-CoV-2: A diagnostic accuracy study, J. Infect. 81 (2020) e75–e78.
[60] O. Ymbern, A. Sohrabi, A. Parandakh, V. Karamzadeh, J. Renault, M. Mirbagheri, Z. Jin, J. Lessard-Wajcer, J. Pimprikar, M. Shen, L. Oliveira, Y. Paschalidis, A. Ng, D. Juncker, 3D printed capillaric chip for instrumentation-free, rapid, and quantitative covid-19 serological testing using saliva, MicroTAS 2020 - 24th International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2020, pp. 1216-1217.
[61] Z. Chen, H. Zhu, D. Malamud, C. Barber, Y.Y.S. Ongagna, R. Yasmin, S. Modak, M. N. Janal, W.R. Abrams, R.A. Montagna, A rapid, self-confirming assay for HIV: simultaneous detection of anti-HIV antibodies and viral RNA, J. AIDS Clin. Res. 7 (2016).
[62] O. Žukovskaja, I. Jahn, K. Weber, D. Cialla-May, J. Popp, Detection of Pseudomonas aeruginosa metabolite pyocyanin in water and saliva by employing the SERS technique, Sensors 17 (2017) 1704.
[63] E.A. Oblath, W.H. Henley, J.P. Alarie, J.M. Ramsey, A microfluidic chip integrating DNA extraction and real-time PCR for the detection of bacteria in saliva, Lab Chip 13 (2013) 1325–1332.
[64] M. Sabalza, R. Yasmin, C.A. Barber, T. Castro, D. Malamud, B.J. Kim, H. Zhu, R. A. Montagna, W.R. Abrams, Detection of Zika virus using reverse-transcription LAMP coupled with reverse dot blot analysis in saliva, PLoS ONE 13 (2018), e0192398.
[65] G. Cabral-Miranda, A.R. Cardoso, L.C. Ferreira, M.G.F. Sales, M.F. Bachmann, Biosensor-based selective detection of Zika virus specific antibodies in infected individuals, Biosens. Bioelectron. 113 (2018) 101–107.
[66] R.W. Peeling, K.K. Holmes, D. Mabey, A. Ronald, Rapid tests for sexually transmitted infections (STIs): the way forward, Sex. Transm. Infect. 82 (2006) v1–v6.
[67] G.W. Lau, D.J. Hassett, H. Ran, F. Kong, The role of pyocyanin in Pseudomonas aeruginosa infection, Trends Mol. Med. 10 (2004) 599–606.
[68] M.-L. Lambert, C. Suetens, A. Savey, M. Palomar, M. Hiesmayr, I. Morales, A. Agodi, U. Frank, K. Mertens, M. Schumacher, Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study, Lancet. Infect. Dis 11 (2011) 30–38.
[69] E.A. Kelser, Meet dengue's cousin, Zika, Microbes and infection 18 (2016) 163.
[70] S. Shukla, S.-Y. Hong, S.H. Chung, M. Kim, Rapid detection strategies for the global threat of Zika virus: current state, new hypotheses, and limitations, Front. Microbiol. 7 (2016) 1685.
[71] S.S. Modak, C.A. Barber, E. Geva, W.R. Abrams, D. Malamud, Y.S.Y. Ongagna, Rapid point-of-care isothermal amplification assay for the detection of malaria without nucleic acid purification, Infectious Diseases: Research and Treatment, 9 (2016) IDRT. S32162.
[72] J. Bhardwaj, A. Sharma, J. Jang, Vertical flow-based paper immunosensor for rapid electrochemical and colorimetric detection of influenza virus using a different pore size sample pad, Biosens. Bioelectron. 126 (2019) 36–43.
[73] X. Yuan, C. Yang, Q. He, J. Chen, D. Yu, J. Li, S. Zhai, Z. Qin, K. Du, Z. Chu, P. Qin, Current and Perspective Diagnostic Techniques for COVID-19, ACS Infect. Dis. 6 (2020) 1998–2016.
[74] L. Azzi, V. Maurino, A. Baj, M. Dani, A. d'Aiuto, M. Fasano, M. Lualdi, F. Sessa, T. Alberio, Diagnostic Salivary Tests for SARS-CoV-2, J. Dent. Res. (2020), 0022034520969670.
[75] J.-M. Zhang, J. An, Cytokines, inflammation, and pain, Int. Anesthesiol. Clin. 45 (2007) 27–37.
[76] Z. Hao, Y. Pan, W. Shao, Q. Lin, X. Zhao, Graphene-based fully integrated portable nanosensing system for on-line detection of cytokine biomarkers in saliva, Biosens. Bioelectron. 134 (2019) 16–23.
[77] J.E. Dimsdale, Psychological stress and cardiovascular disease, J. Am. Coll. Cardiol. 51 (2008) 1237–1246.
[78] A.M. Magariños, B.S. McEwen, Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress, Proc. Natl. Acad. Sci. 97 (2000) 11056–11061.
[79] E.A. Naumova, T. Sandulescu, C. Bochnig, P. Al Khatib, W.-K. Lee, S. Zimmer, W. H. Arnold, Dynamic changes in saliva after acute mental stress, Sci. Rep. 4 (2014) 4884.
[80] M.S. Khan, S.K. Misra, Z. Wang, E. Daza, A.S. Schwartz-Duval, J.M. Kus, D. Pan, D. Pan, Based analytical biosensor chip designed from graphene-nanoplatelet-amphiphilic-diblock-co-polymer composite for cortisol detection in human saliva, Anal. Chem. 89 (2017) 2107–2115.
[81] D. Pan, M.S. Khan, S.K. Misra, Z. Wang, E. Daza, A.S. Schwartz-Duval, J.M. Kus, D. Pan, Based Analytical Biosensor Chip Designed from Graphene-Nanoplatelet-Amphiphilic-diblock-co-Polymer Composite for Cortisol Detection in Human Saliva, Anal. Chem. 89 (2017) 2107–2115.
[82] M. Yamaguchi, Microfluidic Line-Free Mass Sensor Based on an Antibody-Modified Mechanical Resonator, Micromachines 9 (2018) 177.
[83] M.S. Khan, K. Dighe, Z. Wang, I. Srivastava, A.S. Schwartz-Duval, S.K. Misra, D. Pan, Electrochemical-digital immunosensor with enhanced sensitivity for detecting human salivary glucocorticoid hormone, Analyst 144 (2019) 1448–1457.
[84] V. T. U, S. Ghosh, A. Milleman, T. Nguyen, C.H. Ahn, A new polymer lab-on-a-chip (LOC) based on a microfluidic capillary flow assay (MCFA) for detecting unbound cortisol in saliva, Lab on a Chip, 20 (2020) 1961-1974.
[85] K.J. Ducker, R.L. Lines, M.T. Chapman, P. Peeling, A.K. McKay, D.F. Gucciardi, Validity and reliability evidence of a point of care assessment of salivary cortisol and α-amylase: a pre-registered study, PeerJ 8 (2020), e8366.
[86] T. Vinitha, S. Ghosh, A. Milleman, T. Nguyen, C.H. Ahn, A new polymer lab-on-a-chip (LOC) based on a microfluidic capillary flow assay (MCFA) for detecting unbound cortisol in saliva, Lab Chip 20 (2020) 1961–1974.
[87] M.M. Kebede, C. Schuett, C.R. Pischke, The role of continuous glucose monitoring, diabetes smartphone applications, and self-care behavior in glycemic control: results of a multi-national online survey, J. Clin. Med. 8 (2019) 109.
[88] D. Jung, D. Jung, S. Kong, A lab-on-a-chip-based non-invasive optical sensor for measuring glucose in saliva, Sensors 17 (2017) 2607.
[89] V. Srinivasan, V.K. Pamula, M.G. Pollack, R.B. Fair, Clinical diagnostics on human whole blood, plasma, serum, urine, saliva, sweat, and tears on a digital microfluidic platform, Proc. MicroTAS (2003) 1287–1290.
[90] Y. Jia, H. Sun, X. Li, D. Sun, T. Hu, N. Xiang, Z. Ni, based graphene oxide biosensor coupled with smartphone for the quantification of glucose in oral fluid, Biomed. Microdevices 20 (2018) 89.
[91] L.F. de Castro, S.V. de Freitas, L.C. Duarte, J.A.C. de Souza, T.R. Paixão, W. K. Coltro, Salivary diagnostics on paper microfluidic devices and their use as wearable sensors for glucose monitoring, Anal. Bioanal. Chem. (2019) 1–10.
[92] W. Zhang, Y. Du, M.L. Wang, Noninvasive glucose monitoring using saliva nano-biosensor, Sens. Bio-Sens. Res. 4 (2015) 23–29.

[93] L. García-Carmona, A. Martín, J.R. Sempionatto, J.R. Moreto, M.A.C. González, J. Wang, A. Escarpa, Pacifier biosensor: toward noninvasive saliva biomarker monitoring, Anal. Chem. 91 (2019) 13883–13891.
[94] Y. Jia, H. Sun, X. Li, D. Sun, T. Hu, N. Xiang, Z. Ni, based graphene oxide biosensor coupled with smartphone for the quantification of glucose in oral fluid, Biomed. Microdevices 20 (2018) 1–9.
[95] P. Drawz, M. Rahman, Chronic kidney disease, Annals of internal medicine, 162 (2015) ITC1-ITC1.
[96] K.-H. Wang, J.-C. Hsieh, C.-C. Chen, H.-W. Zan, H.-F. Meng, S.-Y. Kuo, M.T. N. Nguyễn, A low-cost, portable and easy-operated salivary urea sensor for point-of-care application, Biosens. Bioelectron. 132 (2019) 352–359.
[97] D. Pandya, A.K. Nagrajappa, K. Ravi, Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension–a research study, J. Clin. Diagn. Res.: JCDR 10 (2016) ZC58.
[98] N. Ahmed, A. Mehmood, N. Dawani, S. Roshan, Salivary urea: a marker for chronic renal disease, Pak. J. Med. Dent. 4 (2015) 03.
[99] E.P. de Oliveira, R.C. Burini, High plasma uric acid concentration: causes and consequences, Diabetol. Metab. Syndr. 4 (2012) 12.
[100] V. Bhole, J.W.J. Choi, S.W. Kim, M. De Vera, H. Choi, Serum uric acid levels and the risk of type 2 diabetes: a prospective study, Am. J. Med. 123 (2010) 957–961.
[101] J. Kim, S. Imani, W.R. de Araujo, J. Warchall, G. Valdés-Ramírez, T.R. Paixão, P. P. Mercier, J. Wang, Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics, Biosens. Bioelectron. 74 (2015) 1061–1068.
[102] S.A. Klasner, A.K. Price, K.W. Hoeman, R.S. Wilson, K.J. Bell, C.T. Culbertson, based microfluidic devices for analysis of clinically relevant analytes present in urine and saliva, Anal. Bioanal. Chem. 397 (2010) 1821–1829.
[103] T.M. Blicharz, D.M. Rissin, M. Bowden, R.B. Hayman, C. DiCesare, J.S. Bhatia, N. Grand-Pierre, W.L. Siqueira, E.J. Helmerhorst, J. Loscalzo, Use of colorimetric test strips for monitoring the effect of hemodialysis on salivary nitrite and uric acid in patients with end-stage renal disease: a proof of principle, Clin. Chem. 54 (2008) 1473–1480.
[104] M. Khan, K. Dighe, Z. Wang, I. Srivastava, E. Daza, A. Schwartz-Dual, J. Ghannam, S. Misra, D. Pan, Detection of prostate specific antigen (PSA) in human saliva using an ultra-sensitive nanocomposite of graphene nanoplatelets with diblock-co-polymers and Au electrodes, Analyst 143 (2018) 1094–1103.
[105] L. Huang, G. Reekmans, D. Saerens, J.-M. Friedt, F. Frederix, L. Francis, S. Muyldermans, A. Campitelli, C. Van Hoof, Prostate-specific antigen immunosensing based on mixed self-assembled monolayers, camel antibodies and colloidal gold enhanced sandwich assays, Biosens. Bioelectron. 21 (2005) 483–490.
[106] D.A. Healy, C.J. Hayes, P. Leonard, L. McKenna, R. O'Kennedy, Biosensor developments: application to prostate-specific antigen detection, Trends Biotechnol. 25 (2007) 125–131.
[107] W.C. Nance, S.E. Dowd, D. Samarian, J. Chludzinski, J. Delli, J. Battista, A. H. Rickard, A high-throughput microfluidic dental plaque biofilm system to visualize and quantify the effect of antimicrobials, J. Antimicrob. Chemother. 68 (2013) 2550–2560.
[108] D.S. Samarian, N.S. Jakubovics, T.L. Luo, A.H. Rickard, Use of a high-throughput in vitro microfluidic system to develop oral multi-species biofilms, JoVE (Journal of Visualized Experiments) (2014), e52467.
[109] P. Panta, D. Andreadis, Introduction to oral cancer, Springer, Oral Cancer Detection, 2019, pp. 1–27.
[110] D. Brandizzi, M. Gandolfo, M.L. Velazco, R.L. Cabrini, H.E. Lanfranchi, Clinical features and evolution of oral cancer: A study of 274 cases in Buenos Aires, Argentina, Age (in years) 40 (2008) 9.
[111] Y.-H. Lin, C.-C. Wu, Y.-S. Peng, C.-W. Wu, Y.-T. Chang, K.-P. Chang, Detection of anti-p53 autoantibodies in saliva using microfluidic chips for the rapid screening of oral cancer, RSC Adv. 8 (2018) 15513–15521.
[112] B.W. Neville, T.A. Day, Oral cancer and precancerous lesions, CA Cancer J. Clin. 52 (2002) 195–215.
[113] T. Dong, N.M.M. Pires, Immunodetection of salivary biomarkers by an optical microfluidic biosensor with polyethylenimine-modified polythiophene-C70 organic photodetectors, Biosens. Bioelectron. 94 (2017) 321–327.
[114] R.C. Page, K.S. Kornman, The pathogenesis of human periodontitis: an introduction, Periodontology 2000 (14) (1997) 9–11.
[115] B.L. Pihlstrom, B.S. Michalowicz, N.W. Johnson, Periodontal diseases, The lancet 366 (2005) 1809–1820.
[116] N. Rathnayake, D.-R. Gieselmann, A. Heikkinen, T. Tervahartiala, T. Sorsa, Salivary Diagnostics—Point-of-Care diagnostics of MMP-8 in dentistry and medicine, Diagnostics 7 (2017) 7.
[117] S. Wignarajah, G.A. Suaifan, S. Bizzarro, F.J. Bikker, W.E. Kaman, M. Zourob, Colorimetric assay for the detection of typical biomarkers for periodontitis using a magnetic nanoparticle biosensor, Anal Chem 87 (2015) 12161–12168.
[118] P. Weselsky, R. Benedikt, Ueber einige Azoverbindungen, Ber. Dtsch. Chem. Ges. 12 (1879) 226–230.
[119] S.A. Bhakta, R. Borba, M. Taba Jr, C.D. Garcia, E. Carrilho, Determination of nitrite in saliva using microfluidic paper-based analytical devices, Anal. Chim. Acta 809 (2014) 117–122.
[120] T. Sorsa, L. Tjäderhane, Y.T. Konttinen, A. Lauhio, T. Salo, H.M. Lee, L.M. Golub, D.L. Brown, P. Mäntylä, Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation, Ann. Med. 38 (2006) 306–321.
[121] T. Sorsa, U.K. Gursoy, S. Nwhator, M. Hernandez, T. Tervahartiala, J. Leppilahti, M. Gursoy, E. Könönen, G. Emingil, P.J. Pussinen, Analysis of matrix metalloproteinases, especially MMP-8, in gingival crevicular fluid, mouthrinse and saliva for monitoring periodontal diseases, Periodontology 2000 (70) (2016) 142–163.
[122] A.M. Heikkinen, T. Raivisto, K. Kettunen, L. Kovanen, J. Haukka, E. Pakbaznejad Esmaeili, J. Elg, D.R. Gieselmann, N. Rathnayake, H. Ruokonen, Pilot study on the genetic background of an active matrix metalloproteinase-8 test in finnish adolescents, J. Periodontol. 88 (2017) 464–472.
[123] A.M. Heikkinen, S.O. Nwhator, N. Rathnayake, P. Mäntylä, P. Vatanen, T. Sorsa, Pilot study on oral health status as assessed by an active matrix metalloproteinase-8 chairside mouthrinse test in adolescents, J. Periodontol. 87 (2016) 36–40.
[124] H.-K. Lee, S.J. Kim, Y.H. Kim, Y. Ko, S. Ji, J.-C. Park, Odontogenic ameloblast-associated protein (ODAM) in gingival crevicular fluid for site-specific diagnostic value of periodontitis: a pilot study, BMC Oral Health 18 (2018) 148.
[125] B.H. Lee, S.H. Kim, Y. Ko, J.C. Park, S. Ji, M.B. Gu, The sensitive detection of ODAM by using sandwich-type biosensors with a cognate pair of aptamers for the early diagnosis of periodontal disease, Biosens. Bioelectron. 126 (2019) 122–128.
[126] J.M. Leppilahti, T. Sorsa, M.A. Kallio, T. Tervahartiala, G. Emingil, B. Han, P. Mäntylä, The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome, J. Periodontol. 86 (2015) 777–787.
[127] M.J. Kivelä-Rajamäki, O.P. Teronen, P. Maisi, V. Husa, T.I. Tervahartiala, E. M. Pirilä, T.A. Salo, L. Mellanen, T.A. Sorsa, Laminin-5 γ2-chain and collagenase-2 (MMP-8) in human peri-implant sulcular fluid, Clin. Oral Implant Res. 14 (2003) 158–165.