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Salivary Theranostics in Pediatric and Special Care Dentistry*

SUMMARY

Saliva as a microfluidic system offers numerous advantages for both general and oral health diagnostic and therapeutic procedures since its assembly is quick, stress-free, inexpensive and non-invasive. Moreover, saliva is frequently referred to as a mirror of the body due to the fact that it can reflect the physiological and pathological state of the body. More than a decade ago the term "Salivaomics" has been introduced with the aim of emphasizing the development of research, knowledge and applications of five salivary constituents: proteome, transcriptome, micro-RNA, metabolome, and microbiome. Contemporary oral health care delivery in pediatric and special care dentistry is focused toward the development of new diagnostic and therapeutical procedures that are essentially non-invasive due to common issue of intolerability to invasive procedures among these patients, with the possibility of increasing participation rates. Besides the criteria of being easily and non-invasive collected, there are additional standards that should be met before routine application in everyday clinical practice; the existence of specific biomarkers for a disease, and ability of having its biomarkers detected using present-day equipment. For example, there are recent suggestions that a salivary RNA panel could objectively differentiate children with autism spectrum disorder from their neurotypical peers. In addition, due to the ease of the administration, the oral cavity is an attractive site for the drug delivery systems development because through this route it is possible to realize mucosal and transmucosal, systemic effect. All these contemporary advances extended the salivary diagnostic approach from the oral to general health pointing towards a promising future of salivary diagnostics for personalized medicine devices.

Key words: Saliva, Theranostics, Pediatric Dentistry

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Introduction

During last two decades a disease pattern substantially shifted from acute and infectious conditions to more complex, chronic disorders. Together with this change, the concept of understanding the disease has moved toward an intensified comprehension of the complicated relationship between causative agents, genes, microbes, environmental factors, nutrition, together with societal determinants as underlying causes of these complex disorders[1]. At the same time, healthcare systems undoubtedly require similar transformation but in this process there are some important challenges and unanswered questions regarding to which extent, when and how it should be performed.

Present-day approach to health, disease, diagnostics and management supports the development of novel diagnostic and therapeutical designs that are in essence non-invasive. The opportunity to evaluate and follow both physiological and pathological conditions, early interfere with preventive and prophylactic measures, examine

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disease initiation and progression, together with monitoring treatment results using non-invasive methods turned out to be one of the most desirable aims for healthcare providers. New technologies are widely employed and readily integrated into contemporary medical systems and without doubt biotechnology is a major power in place to help in healthcare improvement. But, the issue with the access to healthcare, particularly in the most vulnerable groups, such as children and persons with disability still exists. There are numerous reports about unmet health needs, lack of access to proper health care, inability to cope with required medical procedures among these vulnerable groups within general population. There is obvious need to close this existing gap for these populations in gaining access to appropriate health care services that match up with their needs. So far, those who need care the most are every so often the least likely to get it[2-6].

Facing the problem of inequity, disparity and high cost of health care delivery on one side and growing potential with developing high technology medical systems on the other, the World Health Organization recently presented the guidelines which gave the criteria to follow when creating medical appliances, accepted as the ASSURED criteria that pointed towards the need that devices had to be 'Affordable, Sensitive, Specific, User-friendly, Rapid and robust, without employment of complicated Equipment, and be Delivered' to final consumers effectively[7]. Yet to come challenges and potentials of this approach are enormous, and the introduction of simultaneous diagnostics with treatment, commonly referred to as 'theranostics' hold the promise for diagnostic procedures optimization, the therapeutic dose control in the individual patient[8,9]. As clearly pointed out therapeutical approach is no longer focused on the 'average' patient but instead modified for the individual person, known as precision medicine. It has already been shown that personalized medicine, with the use of specially fabricated biosensors, so called 'lab-on-chip' structures, distant monitoring opportunities and microfluidic appliances, will for sure bring significant improvement for the entire healthcare system and will give the chance to the health care providers to be more precise, reliable and to obtain clinically significant data rapidly, all in patients' best interest.

As stated by the report 'Healthy People 2020', oral health is considered as integral to overall health, but according to some observation there is a lack of appropriate interdisciplinary cooperation between the general and oral healthcare providers and patients' misconceptions regarding preventive general and dental care[3,10]. The use of state of the art technologies will able to help benefit patients only in case of efficient integration of various disciplines, with the use of conventional medical approaches together with contemporary theranostic systems and merging of disciplines.

Saliva as physiological fluid

From the physiological perspective, saliva represents a distinctive body fluid continually covering, moisturizing and rinsing the oral cavity and the mucous tissues of the vestibulum and pharynx[11]. It has been extensively examined in cariology, basic oral sciences and dental medicine, but with the introduction of the new concepts of disease managements, the saliva composition and functions require new insight from completely different perspective. Saliva is a clear, slightly acidic liquid, the result of the exocrine secretion of the mucinous or serous salivary glands. The entire oral mucosa contains small salivary glands that are accountable for the secretion of up to 10 % of the whole saliva, while the large salivary glands comprise three pairs of glands (sublingual, submandibular and parotid), producing around 90% of residual saliva. The major constituent, up to 99% of saliva, is water, but also saliva contains a wide variety of constituents: electrolytes, proteins, glucose, urea, ammonia, bacteria, food fragments, blood and epithelial cells[12]. In the oral cavity, the surface cells layers are replaced approximately every 4 hours, while the turnover period for the epithelium is around 4 days[11,12]. Electrolytic component includes the presence of calcium, sodium, chloride, potassium, magnesium, phosphate and bicarbonate. Various proteins of importance for different salivary functions are present in various proportions and they include enzymes, immunoglobulin fraction, glycoproteins, albumins and some oligopeptides and polypeptides[13]. All abovementioned components are responsible for various functions that are attributed to saliva, but the interaction between them gains increased attention, since it appears that is rather specific, controlled and complex.

Two major groups of salivary functions were described: the first is protection of the oral tissues by means of lubrication, antibacterial activity, buffering capacity, enamel remineralisation and tissue repair, while the second is facilitating speech and eating by bolus preparation, enhancing mastication, swallowing, digestion and supporting speech by lubrication of the tongue and lips during movements[14].

Physiological salivary secretion varies between 500 ml up to 2 l during 24 h, while only up to 10 % of the saliva is secreted during night. It has been recognized that the main factor modifying saliva content is the flow index that fluctuates according to the type and level of the stimulus. When salivary flow intensifies, the total contents of protein fractions together with sodium, calcium, chloride, and bicarbonate increase, along with the alkalinity rise, whereas the content of phosphorus and magnesium ions decrease. Analytical salivary testing focus not only on the quantitative determination of the substances present in the saliva, but also on the various physic-chemical properties of importance for salivary theranostics. Saliva rheological characteristics for instance, interfacial tension or surface

dilatational modulus may be helpful in clarification of the interface formed between the liquid and the air and provide additional information regarding the formation of the salivary biofilm in the oral cavity[17]. The pH values ranging from 6.2 to 8 is routinely monitoring in oral health risk assessment. When it comes to the polarity of dielectric materials, saliva could be considered as polar dielectric system. Increased salivary viscosity has already been associated with the high caries risk, but during these studies it has been observed that it is problematic to evaluate salivary flow and viscosity individually and it has been described that the viscosity, defined as the ratio between shear rate and shear stress of saliva, is under influence of shear rate and time, so that saliva was classified as a non-newtonian fluid . Saliva density is in the range of 1.002–1.012 g/ml[11,15,16]. Mucin glycoproteins are for the most part responsible for the elasticity of saliva, together with their important role in the extensional rheological properties of saliva, such as contact angle that can indicate the degree of wetting of saliva on surrounding surfaces.

Salivary composition changes significantly with respect to age, specific physical conditions, particularly in children and persons with disabilities. For pediatric dentistry it is important to keep in mind that there is a significant ascending linear correlation between the age and sodium, protein, immunoglobulin and amylase concentrations, indicating a process of development and maturing of the salivary glands[16]. In various groups of persons with disabilities the pattern of salivary behavior differs. There are reports that excessive salivary flow could be observed in nearly 40 % of persons with cerebral palsy, intellectual and other developmental disabilities. Some groups of disabled increased presence of various substances that significantly differs from general population[11,15-23].

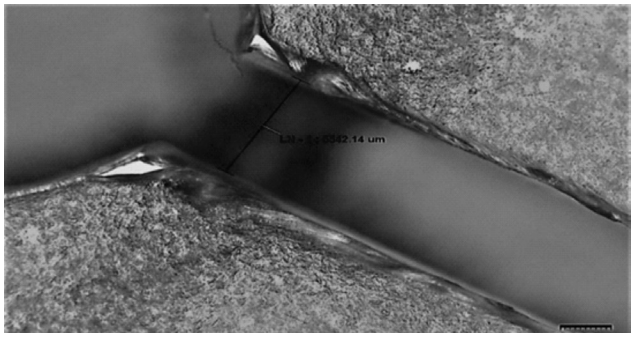

Figure 1. Chip channals measurements using profilometry

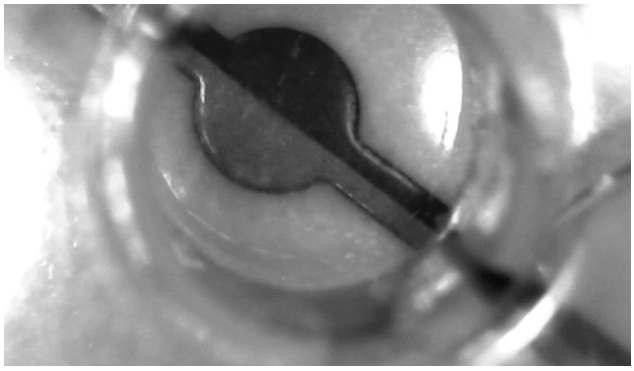

Figure 2. Observational field visualizing laminar liquid flow[34]

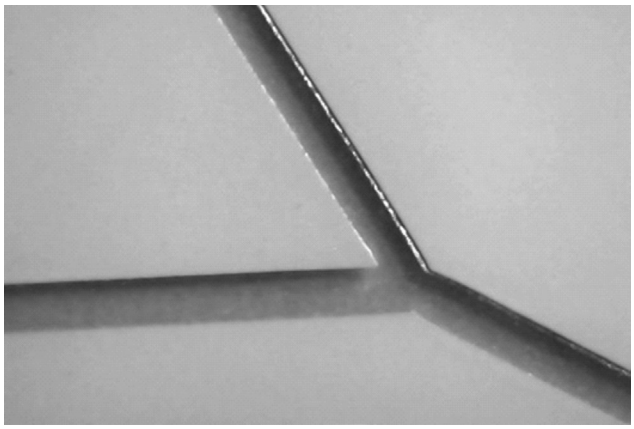

Figure 3. The Y design of PVC chip[34,35]

Microfluidic system in theranostics

Microfluidics can be generally defined as structures controlling the channels with the dimensions in micrometer scale in order to manipulate and handle fluidic samples of a low volume. A microfluidic setup developed with the intention for use in theranostics requires specific design of numerous constituents comprising precise channel construction, sample preparation, substrate choice, control of mixing, combining and reacting (Figures 1-4). All these systems should contain functional, mechanical and electrical components, sensors and actuators. Microfluidic systems offers important advantages including the ability to operate with low volume of analytes, thus requiring lower amounts of expensive reagents., together with the fact that the smaller length scales of microfluidics setups allow quicker analysis and diminished response times[24].

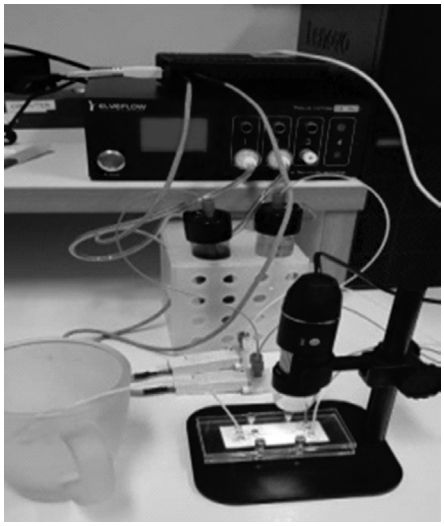

Figure 4. Experimantal setup[34,35]

Various materials were assessed for microfluidic setup applications including, plastics, silicon, elastomers, paper, but the polymeric group of materials, polydimethylsiloxane (PDMS), polyvinyl-chloride (PVC) and polymethyl methacrilate (PMMA) were commercially manufactured to higher extent as a consequence of their lower cost and easier fabrication. Together with the advances of the materials improvements in microfluidic fabrication techniques occurred and the use of xurography, lithography and lasers have enabled appliances with a smaller outline with the reduced expenses[24]. All these simple, cost-effective, and fast fabrication methods have supported the application of various microfluidic setups to a wide range of biomedical areas, but point of care (POC) diagnostics still remains the main application area of microfluidics. The main goal of a POC theranostics studies is to create a chip-based, self- comprehending scale down appliance that can be employed for the examination of numerous, multiplexed analytes in complex substrates, such as saliva[25].

Since increasing number of microfluidic platforms develop for theranostics purposes, the careful selection of material and fabrication method must be taken into consideration, because all medical devices require complex and demanding regulatory approvals, in contrast to research products. And, while there are intensive investigations with the possibility of chip application the reality is more like chip in a lab than lab on a chip[26]. In spite of outstanding progress toward POC clinical systems, only a few completed working prototypes have developed, and there are still important challenges in front the translation of salivary theranostics in clinical practice and the use of already designed setups for everyday use[7,24].

Saliva as diagnostic fluid

Blood specimens are still the most frequently used samples for general health monitoring and specific diagnostic analytes detection[7]. However, blood specimen collection is invasive, can be impractical for people with blood or injection phobia and for those who require day by day monitoring of biomarker levels. In healthcare professionals drawing blood carries the risk for percutaneous injuries and self-contagion, while for the children and persons with disabilities there is constant problem with complying to invasive procedures.

Among the non-invasive organic fluids, saliva is one of the most preferable and practical specimens for general and oral health monitoring as it is readily available and easily collected and stored. In contrast to other biological fluids it "lacks the drama of blood, the sincerity of sweat, and the emotional appeal of tears"[25]. Saliva is frequently called a ''mirror of the body,'' since it can reflect both the physiological and pathological conditions in the entire human organism. It has been clearly pointed that all biological specimens, including saliva could be used as diagnostic samples for diagnostics and control of a disease if they meet the criteria of being easily and non-invasive collected; if they possess specific biomarkers for a disease, and capability of having its biomarkers detected using existing technologies. The term ''Salivaomics'' was introduced in 2008 due to the advances in research about five different ''omic'' constituents of saliva.

It has been confirmed that saliva contains various significant biomarkers. In contemporary diagnostics the term biomarker is used for specific biological or physical characteristics that are indicators of a particular underlying pathological or physiologic state, and in that way biomarkers can be used to evaluate the disease risk, determine the disease severity, and monitor the treatment effects[1].

Discrepancy in the data in regards to the relationship between salivary and blood levels of investigated biomarkers together with the relatively limited studies on saliva and blood correlations offers new possibilities for the research of saliva as a diagnostic tool. The use of saliva as a diagnostic specimen of all health conditions is not likely, but its use with specific diseases remains a possibility and there is a need to clearly specify the scientific and clinical rationale and underlying mechanisms that relate general and oral diseases to saliva.

The future successful translation of salivary theranostics, despite its attractiveness both for the clinicians and researchers is at the moment hampered by several obstacles that need to be addressed before the use of salivary theranostics microfluidic systems becomes clinical reality. First, there is the problem with the biomarker concentration in saliva that is sometimes up to 1000 times less compared to blood or serum, requiring employment of more sophisticated detection technologies[1,27]. In addition, diurnal, circadian, age, gender, diet, genetic, fluid intake related variations of the molecules concentrations require further in depth elucidation, with a particular emphasis on pediatric and special needs populations. Furthermore, the exact pattern of different molecule transportation from blood to saliva is still unknown, and it turned out that majority of the protein present in saliva are of extremely polymorphic nature and continuously going through post translation modifications. Finally, there isn't standardized and adequate saliva collecting method, together with the absence of widely accepted references and callibrations[7,27].

Oral health diagnostics

Salivary diagnostic tests for oral diseases risk assessment are already available in dental offices. But,

considering the opportunities offered by microfluidic systems in terms of higher accuracy, sensitivity and cost-effectiveness it is expected that the introduction of more specific saliva based diagnostic instruments and their integration into specific clinical guidelines will let salivary diagnostics to be used as every day, routine, chair side examinations for numerous oral diseases very soon. Nevertheless, much work still needs to be done before incorporation of saliva based microfluidic diagnostics setups into regular use[28].

There are many approaches in caries risk assessment, some of them are based on analysis of protective factors, while the others focus on pathogen identification. When it comes to analysis of host related properties of saliva its pH, flow rate and buffering capacity are the most frequently analyzed factors in contemporary clinical systems[29,30]. The acidity, pH value, has been recognized as one of the most fundamental nonspecific properties of the cariogenic oral biofilm and surrounding saliva, and significant efforts have been made to integrate pH imaging and pH changes detection into microfluidic platforms. After thorough confirmation, one of the prototypes for monitoring pH changes at the very specific area for the caries development, at the connection interface between dental plaque and of the bacteria present in the saliva has been designed. This microfluidic platform controls flow and chemical concentration environments within the microfluidic channel that enabled the Stephan curve could be investigated on the individual bases, opening the way for the exploration of independent influences to caries development due to acidity assessment confined to a small area, right at the dental plaque enamel interface. In addition, other nonspecific salivary characteristics were employed in caries risk assessment in various groups of persons with disability, and it has been reported salivary viscosity could be related to relatively low caries incidence in persons with Down syndrome, while flow rate could be related to poorer caries protection in persons with cerebral paralysis. The majority of caries risk diagnostics test investigates the presence and number of cariogenic bacteria, Streptococcus mutans and *Lactobacillus*, with microfluidics offering the possibility of including specific, bacterial genomic, proteomic, metabolomics and transcriptomic approach witin the same context. So far, it was only possible do detect the presence and determine the amount of cariogenic bacteria, but there are new systems that could provide additional information regarding the cariogenity of the bacterial biofilm. It has been reported that the low concentrations of alpha defensing in saliva play a role in higher caries experience in children, whereas higher content of salivary mucin stimulates agglutination of *Streptococcus mutans*. Kaczor-Urbanovic[30] described the possibilities for salivary based diagnostics of infectious diseases that could be possible applied for cariogenic bacteria. This “lab-on-a-chip” system for detecting bacterial pathogens contains the specimen collector, plastic microfluidic cassette chip for saliva processing with immunochromatographic assay[30]. In addition, candidiasis infection biomarkers have been already diagnosed in saliva. Despite this improvements and specific designs, there is still no single salivary test that has presented reliability and precision caries risk assessment, and because of that it has been recommended that a combination of known risk factors should be used in order to determine persons who are at higher risk for caries occurrence which is explained by the participation of numerous local and systemic risk factors in the caries development[28].

Similarly to caries risk assessment, the analysis of saliva may help as a valuable instrument in microfluidics based toolboxes in the assessment of the periodontal diseases risk, the disease status description, evaluation of the response to treatment and prediction of disease progression. Unfortunately, periodontal disease risk assessment translation into microfluidics setups share same challenges with caries. In order to overcome these issues, both researchers and clinicians need to define and determine the specific set of reliable biomarkers closely related to the area of interest. In periodontal disease, these biomarkers include genetic material and various proteins, together with the biomolecules present in the gingival sulcus fluid, periodontal pocket and saliva[27,28].

In the field of orthodontics there are mouthing suggestions that saliva could be used as a diagnostic tool to examine the risk and the development of root resorption during orthodontic treatment[30].

General health diagnostics

Children with chronic diseases and persons with disabilities are frequently scattered between various specialists, all of them performing independent diagnostic test. The introduction of new, multiplexed and reliable diagnostic procedures that are standardized and could be shared between various physicians could decrease the number of unnecessary visits, decrease the costs and improve the compliance rate in these groups of patients.

In pediatric and special care dentistry, in persons with ADHD, autism, anxiety disorders, intellectual disability and dental phobia, valuable information could be obtained by assessment of the changes in hormones such as cortisol, alpha-amylase and glutathione. Since testosterone in saliva is free, unbound with proteins, the use of saliva for its determination is completely justified and recently, determinations of testosterone levels are widely used in evaluating the extent of aggression, depression, violence, and antisocial behavior in psychiatric patients. At the moment salivary diagnostics relies on examining of the rhythm hormone excretions, and the function of endocrine system has been evaluated employing dynamic tests that control not only the

concentration but metabolism of hormones as well. This approach can be used in evaluation of secreted hormones, but also in analysis of hormones used as medications in hormone replacement therapy, and the joining of these methods could extensively reduce the costs of treatment and bring additional risk management in various dental procedures in these patients. Similar test strips are widely employed for a variety of applications, such as home pregnancy tests and detection of substance abuse.

Numerous viral infections such as hepatitis A, B, C, Epstein Barr virus and herpes have their detectable biomarkers in salivary samples. Measuring the level of salivary antibodies enables detection of these infections with high specificity and sensitivity (nearly 95%)[30]. The majority of these infections have oral symptoms and manifestation, and there is reciprocity between general and oral health status, a clinical challenge that requires further clinical and research elludication.

Besides oral cancer, saliva serves as a valuable diagnostic fluid for the early detection of different cancers such as breast cancer, pancreatic cancer, lung cancer and gastric cancer. In patients with cystic fibrosis increased levels of prostaglandins and decreased concentrations of protease enzyme were recorded in saliva. In addition, an extensive variety of stressors have been investigated in occupational and environmental medicine.

Some wide spread autoimmune diseases are relatively frequently seen in dental office and saliva offers important opportunity as a tool in detection of Sjogren syndrome, cystic fibrosis and celiac disease. All of the abovementioned disease have specific biomarkers detected in saliva, but it should be noted that early symptoms of these conditions can be objectively detected during routine dental clinical examination, such as the presence of aphtous ulcers in celiac disease or prolonged xerostomia in Sjogren disease[30].

Unfortunately, the comorbidity of disability and numerous psychiatric and neurological conditions is extremely prevalent. Cortisol and alpha amylase are extensively assessed with this respect since it has been demonstrated that their concentrations in saliva specifically fluctuate in persons with anxiety disorders. Salivary testosterone is used in evaluating the level of depression, violent and antisocial behavior in psychiatry. Furthermore, salivary biomarkers are used in Alzheimer's disease and the increase of TAU proteins has been related to this neurodegenerative condition[30]. The interaction between periodontal disease and Alzheimer's gains increasing interest of the oral health researchers, where salivary based microfluidic setups could offer tremendous possibilities. It has also been demonstrated that salivary protein, DJ-1 could be a marker of Parkinson's disease progression and according to the preliminary report salivary biomarker analysis holds a promise for reliable evaluation of dopaminergic function in persons with Parkinson's disease[31].

Saliva is valuable sample in pharmacology since it enables evaluation of the therapeutic drug levels, as well as treatment outcomes, detection of overdose and analysis of the biochemical and physiological effects of various medications such as carbamazepine, cisplatin, diazepam, digoxin, ethosuximide, irinotecan, lithium, metoprolol, paracetamol, phenytoin, primidone, procainamide, quinine, theophylline, or valproic acid. Also, cotinine can be analysed in saliva of smoking subjects. In this way, it can serve as a diagnostic and control sample in many scientific and clinical disciplines such as medicine, dentistry, forensics, biochemistry and pharmacology. Saliva is a useful diagnostic tool in forensic sciences, where there is a possibility to differentiate individuals, who are still alive, from dead bodies[30].

Oral drug delivery

The oral cavity presents a practical, safe, and very appealing site for medication delivery with good tolerance and compliance by patients. The mucosa of oral cavity is moderately permeable, exhibits the potential for short recovery time after trauma, demonstrates the tolerance to possible allergens, and it has good vascularization[32]. Within this context, drug delivery via oral cavity is categorized into three groups, sublingual delivery, which is in general a systemic delivery of medications through the mucosal tissues covering the floor of the mouth, buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa), and local delivery, which is drug delivery into the oral cavity[32].

Today, a number of polymer-based delivery systems like fibers, films and strips are used to deliver a variety of drugs in oral cavity[33-35]. They, as the majority of oral drug delivery systems, use chemical or physical released control to adjust the release rate, which is rather limited compared to an active, mechatronic drug delivery system, and many therapeutic challenges still remain, including the difficulty in obtaining adequate concentration of the drug at the target area during time and simultaneous detection of the biomarker and timely drug release.

The final goal for salivary theranostics systems would be integration of the microfluidic setups with oral tissues or intraoral appliances and some of these devices have already been introduced. Key problems towards the effective accomplishment of applicable wearable biosensors detector and drug delivery systems are related to materials choice, operation mode, analytical tests, interaction and data collection, processing, and safety. Even though reliable and precise bisensors exist for several decades, the materials, fabrication techniques are often incompatible for realizing their wearable counterparts[32,36].

Local delivery to tissues of the oral cavity has a number of applications, including the treatment of acute or chronic pain, bacterial, viral and fungal infections, aphthous ulcerations and dental stomatitis, and the facilitation of tooth movement with the use of prostaglandins[36]. Consequently, studies on the release of antimicrobial agents, e.g., chlorhexidine, tetracycline, or metronidazole, from several polymeric systems, and the evaluation of their clinical effects have been reported. Novel oral dosage forms consist mainly of sustained release systems for oral mucosal delivery intended to release the drug within a defined period of time. They describe the use of adhesive patches, stripes, polymers for slow release and chewing gum, microfluidics offers significant improvements in the field The choice of better oral bioadhesive dosage forms also depends on the characteristics of the drugs and on the site to be treated (periodontal pocket, gingiva, teeth, cheek mucosa, or systemic).

Mouthguard biosensor with telemetry system for monitoring of saliva glucose has been successfully designed and integrated into wearable intraoral appliance, where electrodes are formed on the polyethylene eterephthalate glycol (PETG) surface of the mouthguard[37]. The mouthguard biosensor will be useful as a new approach for immediate non-invasive glucose level examining for improved and safe treatment in dental office.

There is a need to develop sensors that are small and light for seamless integration with the human body for daily life. In order to equate the mechanical properties of the device with that of the tissues, researchers have developed highly flexible plastic and textile-based wearable chemical sensors that can detect electrolytes, metabolites or volatile organic compounds[36]. For example, one must detect a whole host of chemical as well as physical parameters simultaneously for complete profiling of person's well-being. Such multiplexed detection commands the wearable devices to include a high density of individual, miniaturized chemical sensors on a single, small platform.

Conclusions

Salivary collection methods and biomarkers need to be standardized and validated. Also, new assays and devices need to be developed at a commercially feasible rate. Microfluidic setups offer the possibility of application in salivary diagnostics, but it is necessary strictly to follow the parameters of the experimental conditions and precisely define physico-chemical biomarkers. Controlled drug delivery for routine use in dental clinical practice utilizing microfluidic setups require additional preclinical confirmation, calibration of all relevant parameters and the improvement and merge of existing medical and engineering technologies.

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