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Biomarkers in Medicine

Science of interdisciplinary salivary bioscience: history and future directions

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Salivary bioscience is noteworthy in its history, as well as in the breadth and scope of its impact. The minimally invasive nature of sampling oral fluid allows for evaluation of individual and intra-individual change in biological processes in ways and settings not possible with traditional biospecimens. The range of measurements is expansive (e.g., DNA, hormones, cytokines, antibodies) and modern technologies enable simultaneous multisystem assessment from a singlet specimen. Used in combination with modern multivariate analytical models, the capacity to repeatedly assess multisystem and level measurements collected from the same individual over time enable operationalization, testing and refinement of complex biobehavioral models. This review describes the emerging narrative of salivary bioscience, and aims to inform and reveal opportunity for innovation and discovery.

Plain language summary: Oral fluid collected from humans and animals that can be used in medical and research settings has a rich history of development and represents the growing field of salivary bioscience. This is in part due to the ease of oral fluid collection (for example, no blood draw necessary), which allows researchers and clinicians the opportunity to evaluate how individuals differ in biological processes both over time and how they compare to other individuals (the same can be said for animals), while using a minute amount of liquid to do so. The ease of collection and modern technology allows these samples to be collected at multiple time points and in places where this was not possible previously, like in the home. Modern technology has made it possible to use a small amount of oral fluid to assess many different biological measurements, including ones associated with hormones, infection and stress. This review provides more information about the history, innovations, and future potential of oral fluid in research and medicine.

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Keywords: interdisciplinary integration • oral fluid • salivary biomarkers • salivary bioscience

The past few decades have witnessed the emergence of a new field of scientific inquiry – interdisciplinary salivary bioscience (ISB). Its students – largely from disciplines focused on explaining individual differences in human health, behavior and/or development, as well as using oral fluid (i.e., oral fluid not specific to gland secretion) for diagnostic/clinical purposes – are empowered by a collective effort that integrates knowledge derived from basic oral biology with advances in technology to make monitoring of biological processes in oral fluids and application to various scientific fields (e.g., behavioral sciences, infectious disease, drug abuse, oncology) possible. So far, ISB has been noteworthy in its history, and in the breadth and scope of its scientific impact. Online citation indices reveal that within the last 20 years the pace of scholarly productivity in this field has increased to more than 1000 empirical articles per year. The breadth and depth of impact is largely derived from the minimally invasive nature of sampling – allowing evaluation of individual differences and intra-individual change in biological processes in ways and settings (e.g., home, work, school, play and the laboratory) not possible with traditional biospecimens (i.e., blood, urine, tissue). The impact also results from programmatic efforts that advanced our understanding of the constituents in oral fluids. Today the range of measurements possible from oral fluid specimens includes nucleic

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acids, epigenetic markers, enzymes, hormones, environmental chemicals and elements, disease-specific antibodies, drugs and their metabolites, microbes and the metabolome, and cytokines.

Milestones, historical markers & vista points

A number of scientific milestones contributed to expansion in the scientific focus on oral fluid beyond its basic role in oral biology. The first are studies (circa 1980) revealing that small molecule stress-related and reproductive hormones (e.g., cortisol, testosterone, estradiol, progesterone) were measurable in oral fluid, that levels in saliva exhibited strong association with levels in serum/plasma, and that hormones in saliva reflect the 'free' or noncomplexed form of these molecules rather than the complexed forms [1]. Studies now routinely employ salivary hormones in basic, descriptive and clinical investigations, and entrepreneurs have developed consumer applications available to the public. A second milestone occurred (circa 1990) in response to the need for a surveillance tool for HIV serostatus while avoiding the risk of transmission by accidental needle stick. This critical need resulted in the creation of the first commercially available point-of-care salivary immunodiagnostic assay for an infectious disease (OraQuick HIV 1/2, OraSure, PA, USA). Since that time, the sensitivity and specificity of many salivary assays for infectious diseases has been established (e.g., [2]). A third event was the US Surgeon General's first report on oral health, linking poor oral health and risk for chronic disease (e.g., diabetes, cardiovascular disease) [3]. The report also focused attention on salivary measures as intrinsically important in their own right – not just as surrogates of their circulating counterparts (i.e., measures of systemic biological activity). A fourth significant milestone was characterization of the salivary proteome, revealing 1000+ analytes to be present in oral fluid and setting the stage for consideration of saliva as the diagnostic fluid of the future (see Malamud and Niebala [4] for review). In addition, the ability to obtain a high quality and quantity of DNA (e.g., [5]) as well as metabolites of therapeutic drugs (e.g., [6]) and drugs of abuse (e.g., [7]) from oral fluid samples has had significant impact in psychiatry, forensics, medicine and even consumer applications. As the narrative evolved over the last 30 years, the field worked to establish standards for sample collection, handling, transportation and storage; how to optimally measure salivary analytes, including those in lower concentrations that require sensitive technology for detection; and how to harmonize and standardize salivary assays to immunodiagnostic industry criterion to ensure measurement validity, precision and reproducibility (e.g., [8,9]). This had led to the most recent milestones in oral fluid work: the application of salivary testing by commercial entities (e.g., 23 and Me, www.ancestry.com) for consumer reports on genetic and health-related markers, and the application of oral fluid for SARS-CoV-2 (COVID-19) testing.

Interdisciplinary by nature

The integration of ISB into models of human behavior, health and/or development often is executed most effectively by teams of investigators each with overlapping interests but also nonredundant expertise (e.g., psychologists, biologists, statisticians, social ecologists). A primary reason for the team science approach is that Principal Investigators (PIs) rarely have the luxury of pausing their research programs to retrain, and the depth of biological knowledge required to be competitive is nontrivial. One consequence of this interdisciplinary team science approach, and the perception that authors would be better served for merit and promotion (tenure) to publish works in journals of their primary discipline, is that 'the body of work' that defines the field of salivary bioscience is widely scattered throughout the literature in many different field-specific journals. Interdisciplinary working environments can generate synergy that accelerates organic scientific advances but can also be frustratingly slow going and challenging (see Stokols *et al.* [10] for a review). For young investigators, in particular, working in interdisciplinary teams can present risk if their departmental home does not place value on contributions they make in collaborative efforts. These challenges may partially explain why it has taken time to recognize ISB as a field. The current push toward more cross-disciplinary research collaborations and interdisciplinary approaches to clinical care may provide an avenue for further expansion of ISB.

High-impact innovation & discoveries across disciplines

There are a number of applications for oral fluid assessment in medicine, biology, oral health and biobehavioral research and clinical work. Indeed, a plethora of original empirical contributions across fields have incorporated oral fluid measures, with rising publication numbers seen yearly and a noticeable uptick associated with the COVID-19 pandemic (Figure 1). In psychology, most of these studies focus on the major psychobiology components of the stress response via assessment of the hypothalamic–pituitary–adrenal axis, using salivary cortisol (i.e., unbound cortisol), or the sympathetic branch of the autonomic nervous system, using salivary alpha-amylase, or both. Using only these

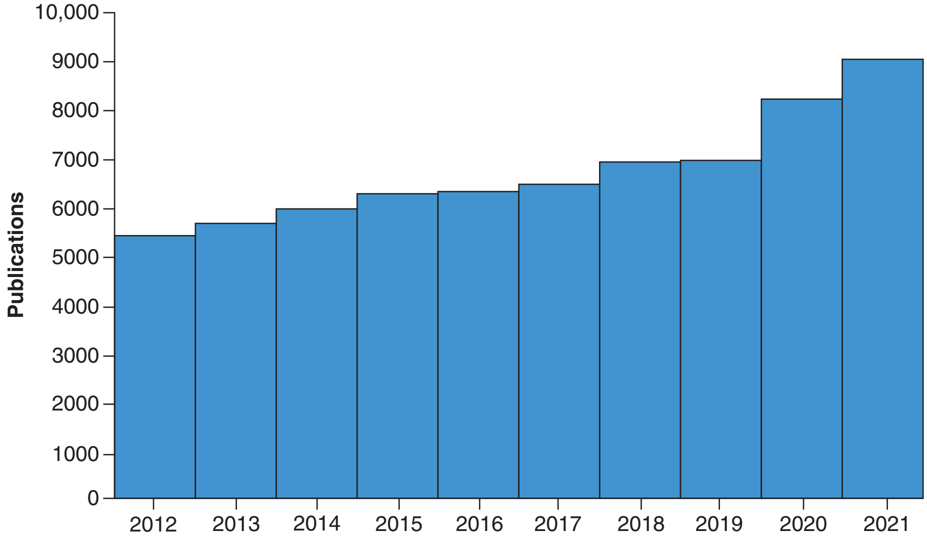

Figure 1. Number of publications from 2012 to 2021 that included 'saliva', 'salivary' or 'oral fluid' as a keyword via PubMed.

two, of the many salivary bioscience tools, psychological science has investigated high-impact research questions linked to several key theories and hypotheses: the context contingency effect (e.g., [11]), biological susceptibility and sensitivity to context (e.g., [12]), the biopsychosocial model of the family (e.g., [13]), effects of adversity and toxic stress (e.g., [14]), risk versus resilience (e.g., [15]), adaptive calibration (e.g., [16]), social evaluative threat (e.g., [17]), and developmental origins of health and disease (e.g., [18]).

ISB studies have focused on pregnancy, infancy, childhood, adolescence, young adulthood, middle age and the elderly; some take place in the lab, at home, work, play and in extreme environments (e.g., space, solitary confinement, Antarctic, competition); and involve diverse participants from normative, at-risk, psychiatric, elite athlete and military (warfighters, sailors, submariners, astronauts, special forces, pilots) groups. At the intersection of salivary bioscience and stress research, eliminating the need for a phlebotomist while providing a less stressful means of providing samples (i.e., no venipuncture or indwelling catheter), has allowed ecological momentary assessment work that incorporates oral fluid biomarkers like cortisol to flourish (see Mehl and Conner [19] for an overview of Ecological Momentary Assessment and cortisol). A noteworthy theme across studies is that context is a powerful determinant of the expression of individual differences in levels of biological variables, and that context is a potent moderator of the expression of relationships between biological processes and outcomes (e.g., health, behavior, cognition) of interest (e.g., [20]).

Applications of oral fluid assessment in genetic-based research have also grown over the years. The willingness to adopt oral fluid sampling may be partially attributable to the ease of assessment, where miniscule volumes of oral fluid (100 μl) can be used to extract human DNA and determine genetic polymorphisms (such as the genetic variations associated with common psychiatric disorders or development of chronic conditions), as well as determine epigenetic markers (i.e., methylation) and markers of cellular aging (e.g., telomere length). Microbial DNA can also be isolated and used to characterize the oral microbiome and metabolome (e.g., [21]) – a salient opportunity given the intense focus at the NIH on the microbiome. Moreover, modern kits now allow for oral fluid collection specific to genetic analyses that can be stored at room temperature for 5 or more years without significant DNA degradation, permitting application of biomarker assessments at future time points past initial collection. A recent large-scale application of diagnostic oral fluid was employed by the Screen Project that offered genetic screening for breast cancer via detection of *BRCA1* or *BRCA2* mutations to Canadians and demonstrates the current application of oral fluid work in genetics and cancer, and the potential future avenue of direct-to-consumer testing [22].

From less than an eye dropper droplet of oral fluid, disease-specific antibodies can be determined to indicate whether an individual has been exposed or not exposed to an infectious agent (e.g., waterborne, foodborne, respiratory and sexually transmitted diseases); and examine antibody production in response to pathogen exposure, viral reactivation or vaccination is influenced by stress, adversity and health behaviors (e.g., [23]). Perhaps of most immediate interest, oral fluid assessments are being utilized for SARS-CoV-2 work, with noted promise for surveillance and prevalence studies (e.g., [24]) and antibody monitoring following vaccination [25]. Salivary samples have been shown to be comparable to nasopharyngeal swab testing for active SARS-CoV-2 infection [26] and yield comparable results to plasma when processed for SARS-CoV-2-specific IgG, demonstrating the diagnostic utility of oral fluid as a means to widely accessible testing for SARS-CoV-2 infection and antibodies as part of larger public health strategy [27,28]. Moreover, a recent systematic review and meta-analysis supports saliva testing for COVID-19 as a more cost-effective, reliable point-of-care measure for COVID-19 that may replace the need for nasopharyngeal sampling [29].

Small volumes of oral fluid can also be used to assess environmental chemical exposures (e.g., [30]). For example, cotinine, a metabolite of nicotine, is very precisely assessed in saliva and has been used to index exposure to second-hand tobacco smoke (e.g., [30,31]). Exposure status may be difficult to establish when markers of environmental exposures are cleared quickly by the body or the exposure itself is infrequent [30]. In either case, a single time point sampling strategy may yield a false negative. In this case, repeated self-sampling at home of oral fluid can occur with minimal participant burden and increase the probability of exposure detection.

Sleep-, psychosocial- and pain outcome-based research represents another growing field where salivary work can prove beneficial, as sleep disturbance and sleep loss is associated with increased stress, progression of chronic conditions (e.g., coronary heart disease) and increased pain severity (e.g., [32,33]), and multiple salivary markers have been suggested for future pain work (e.g., [34]). Salivary melatonin measured repeatedly prior to, and after, dark onset (until bedtime) in the home is a key tool for healthcare professionals focused on the diagnosis of circadian rhythm sleep disorders (e.g., [35]). Further, dim light melatonin onset computed from repeated salivary melatonin assessments is used to prevent patients from receiving treatment at the wrong circadian time and may improve future sleep disorder diagnostics.

In the clinical realm, noncompliance with repeated venipuncture can be a significant barrier to effectively monitoring therapeutic drugs levels. One area of medicine where this is particularly relevant is for psychiatric medications, like lithium, that have narrow therapeutic windows – if the dose is too low treatment effects are minimal, but if the dose is too high iatrogenic effects (lithium toxicity) can be life-threatening [36]. Investigation of the utility of salivary monitoring of lithium in psychiatric patients is underway. Beyond psychiatry, oral fluid assessments in the clinical and/or emergency medicine settings are useful for monitoring drug abuse both for a wide range of prescribed medications (e.g., [37,38]) and illicit substances (e.g., amphetamines, cannabis, cocaine, opioids) (e.g., [39,40]). With regard to illicit drugs, a recent systematic review supports an association between drug levels in urine and oral fluid, with oral fluid indicated as potentially more useful for immediate marijuana detection [41]. However, it should be noted that drug-dependent changes (e.g., in salivary pH, flow-rate) can affect drug detection, thereby limiting the utility of oral fluid for some drug monitoring (see Thomas [6] for review).

In some of our most recent work, levels of uric acid (UA; a product of purine metabolism and potent anti-oxidant) and osteocalcin (a bone-derived hormone) in saliva have been shown to have strong correlations with their circulating counterparts. Briefly, UA regulates the activity of the renin–angiotensin system (a system which modulates blood pressure) and a high-impact recent report suggests that osteocalcin regulates reactivity of the parasympathetic branch of the autonomic nervous system to fear (e.g., [42]). Research programs focused on individual differences in the reactivity and regulation of the stress response would seem well-served by inclusion of oral fluid measures of variation in the renin–angiotensin system and parasympathetic branch of the autonomic nervous system in addition to those for the hypothalamic–pituitary–adrenal axis and sympathetic branch of the autonomic nervous system (see Lucas *et al.* [43]).

Behavioral neuroscientists are interested in oral fluid because, among other things, it is known to contain the presence of central nervous system proteins (see Thomas [6] for review). Salivary measurements (e.g., GFAP) are being explored as biomarkers for onset and recovery from traumatic brain injury and blood–brain barrier disruption (e.g., [44]), as well as for neurological and neurodegenerative disorders (e.g., [45,46]). After an extensive review of the literature, Thomas concludes there is "*exciting potential for oral fluid to be translated into clinical diagnostic, prognostic, and screening efforts for neurological disorders*", but extensive validation is needed in large cohort studies to realize that potential [6] .

Poor oral health is a silent epidemic in North America [3]. Oral health status varies by region, socioeconomic status, age, sex, race and ethnicity, and is strongly associated with risk for chronic disease. Proinflammatory cytokines (IL-1b, IL-6, TNF-a, IL-8 and others) and markers of tissue integrity (MMP-8) can all now be very precisely measured in oral fluids (e.g., [47]) and easily applied to oral health research. Many of these markers, multiple cytokines in particular, can be assessed simultaneously in the same sample using multiplexing assay technology. Moreover, emerging periodontal research has shown the possible utility of oral fluid sampling to assess MMP-8 as a point-of-care test for periodontal disease [48]. There is direct relevance to the national research agenda (i.e., see National Institute for Dental and Craniofacial Research), and reason to believe there are reciprocal effects between oral health and behavior, and clear pathways for scalable behaviorally focused interventions with high probability of improving quality of life.

In addition to the numerous applications for oral fluid assessment in human research, oral fluid sampling is also utilized in animal research and veterinary medicine. For example, salivary cortisol sampling is commonly used for assessing stress in pigs and domestic canines, and is more recently being used in nonhuman primate cortisol research as well [49–51]. In a recent systematic review of canine literature, saliva was a common sampling type for proteomics assessments [52]. Moreover, Iacopetti and colleagues previously provided an overview detailing the use of saliva sampling in canines to assess immunoglobulins, rabies virus antigen, drug monitoring and C-reactive protein [53]. It is important to note that animal health can greatly affect human health whether this be in the context of farm animals, domesticated pets, or in assessment and/or monitoring of cross-species health concerns. The continued adaption of salivary assessments in the animal world is growing and may provide a noninvasive assessment tool important for the One World One Health approach that is currently recommended by multiple international organizations (e.g., WHO, World Organization for Animal Health).

Specialized strategy & tactics

The opportunity to collect multiple samplings without placing high burden on the sample donor or changing the ongoing flow of the social ecology has enabled innovative research design and called for unique analytical strategy (see Riis *et al.* [54] for review). A common design involves collecting oral fluid multiple times prior to, then multiple times following an acute event. In these studies, investigators monitor indices reflecting individual differences in reactivity and recovery, using metrics such as area under the curve. Moreover, many analytes measured in oral fluid show distinct diurnal patterns of production and collecting samples multiple times across the day on multiple days is also common. Metrics derived in this type of sampling design reflect analyte changes in response to waking and total production across a day, as well as the diurnal slope across the day. This approach is often used when studying the impact of chronic stress, or pre- and post-intervention.

Oral fluid sampling has also been employed in the context of everyday life as a complement to ecological momentary behavioral assessments (e.g., [19,55]). The ease of collecting multiple measurement time points allows advanced statistical techniques to be applied that separate distinct sources of variance. Several recent works explore the utility of latent-state trait modeling to extract and explore the correlates and concomitants of a trait-like component of the variance in salivary testosterone [56], cortisol [57], UA [58] and alpha-amylase [59]. An intriguing series of studies suggest levels of salivary biomarkers are correlated between dyads sharing common social experience – between mothers and their infants (e.g., [60]), romantic couples (e.g., [61]) and best friends (e.g., [62]). Expansion of the research focus beyond the individual level of analyses raises fascinating opportunity for dyadic-level analyses of biobehavioral associations. Further advancing our understanding of how attunement of these measures is moderated, established, maintained and re-established after interruption seems well worthwhile. Given the importance of social context in the expression of biobehavioral relationships (e.g., [20]), the integration of salivary bioscience methods with advanced social network modeling is being used to explore biological underpinnings of social connections in organizations (e.g., [63,64]).

Special considerations & potential limitations

There are a number of considerations for PIs and laboratory technicians when measuring biomarkers with oral fluid that will both inform study design and strengthen the resulting biological data. For oral fluid assessments to reach full research potential, the following should be accounted for.

Special considerations

The types of samples obtained and the sampling method should be at the forefront of PI consideration at each portion of the study, from inception of the study design and aims, to laboratory assessment and eventual data dissemination. For sampling type, blood and oral fluid samples provide different information for some biomarkers (e.g., some cytokines, see Riis *et al.* [65] for full review). Hormones provide an excellent example of this, where salivary levels indicate the 'free unbound fraction' or biologically active fraction in the general circulation [66]. It is paramount to understand the sample type that was collected, as the range for the biomarker levels will vary between blood and oral fluid, and so should data interpretations. An additional example of sample type importance can be seen in microbiome research, where fecal sampling reflects the gut microbiome and saliva sampling assesses the oral microbiome; although both are associated with systematic health, PIs should be aware of key differences when planning their study design.

Sampling location, collection device, timing for sample collection and sample handling are all important considerations for work involving oral fluid. Whole saliva is composed of a number of oral fluid subtypes (e.g., submandibular saliva 65%, sublingual saliva 4%), and some biomarkers will vary by these subtypes, meaning it is important to consider where in the mouth a sample is being collected from for particular biomarkers (see Hernandez and Taylor [67] for full review). Various collection techniques can be used to obtain oral fluid samples; during study development PIs should pay special focus to their biomarkers of interest (e.g., sampling location, volume needed) and intended population (e.g., species, age, ease of sample production) needs.

Collection device can refer to variations of passive drool, swab, microsponge or filter paper collection, each with their own advantages and disadvantages. For passive drool (largely considered the gold standard), participants salivate freely into a collection tube sometimes with the aid of a straw or mouthpiece. This collection method tends to be cost-effective, allows for collection of large sample volumes in a relatively short amount of time, eliminates the need for swab and/or mouth rinse and produces the most versatile sample for biomarker analyses [68]. Collection swabs can come in many different forms: swabs contained in tubes are easy for participants to use and have excellent recovery, but may restrict the potential biomarkers available for analyses, and attention to where the swab will be placed in the mouth is necessary. For children or others where these two methods may prove inadequate, a microsponge may be considered as it is easy to handle and collect with, though there is the potential for evaporation of the sample and the aforementioned biomarker restrictions with swab collection remain true. Perhaps of special interest for animal research, extended-length oral swabs and filter paper exist that can allow for easier sampling, though other methods may still be applicable (e.g., rhesus monkeys can provide passive drool [50]).

In addition to these considerations, the time of day that samples are collected and the timing between each sample collection (in the case of multiple assessments) are of utmost importance for some biomarkers. Perhaps one of the best examples of this is salivary cortisol: since it follows a circadian rhythm for production, cortisol levels follow a predictable pattern with a peak 1 h post-wakening and reaching lowest concentrations at night. However, for PIs interested in acute stress responses via cortisol assessment, multiple measurements can be obtained pre- and post-stressor (see Smyth and Clow [69] for full review).

Furthermore, once samples have been obtained, the integrity of the samples and resulting data can be greatly affected by sample handling. PIs and research associates who will be assisting in the collection, storage, transportation and/or laboratory processing of samples should be aware of temperature recommendations. While these recommendations may vary according to the biomarker(s) being included (e.g., see Riis *et al.* [9]), best practice typically suggests that oral fluid should be immediately frozen at ≤ 20 °C and at -80 °C for long-term storage to avoid sample degradation and bacterial growth [68]. Additionally, the number of times a sample is frozen and thawed (i.e., freeze–thaw cycles) can negatively impact sample integrity [9,68]. PIs can work with laboratory staff when planning study execution to determine if aliquots should be obtained from samples prior to long-term storage, and to ask that all freeze–thaw cycles be denoted by the lab, as this may explain sample variability and/or viability.

For analytes of interest that are released by salivary glands (e.g. salivary alpha amylase) and large bloodborne molecules (e.g., salivary dehydroepiandrosterone i.e., DHEA), it is vital to collect flow rate information so this can be accounted/corrected for, as these analytes' levels are dependent on flow rate (see Hernandez and Taylor [67] for full review). To assess flow rate, the time it took a participant to produce a specific sample volume should be recorded. This is very simple for passive drool sampling, where only the collection length of time will need to be denoted for flow rate calculation. For swab collection, time to obtain oral fluid collection will be needed, in addition to the weight of the collection device before and after collection. It is important to note that when a swab is being used, participants should be asked to remove the swab at full saturation, or misestimation of the time for sample collection could occur and result in inaccurate flow rate. Additionally, device weight does not necessarily need to be determined during collection. Instead, an empty collection device can be weighed by the lab, and samples can be weighed before analyses are conducted; however, collection time must be collected during the run of the study.

Although not exclusive to testing with oral fluids, there are some wet laboratory considerations that may be helpful to PIs and laboratory technicians who are new to these types of measurements. These include temperature in the laboratory, consistent pipetting, proper assay handling (reagent temperature, assay timing), proper pipet calibration, sample dilution for biomarkers in low concentrations and potential staff inconsistencies that could introduce error. When samples are being run, best practices would be to denote any differences in a particular assay run or changes in the laboratory environment or staff that could impact results. Lastly, specialized statistical techniques have been explored for salivary analytes and should be considered when at the data-cleaning and -analysis phase [54,70–72].

Potential limitations

With assessments of oral fluid, there is an inherent risk of blood contamination in the sample, whether it be due to poor oral health, oral injury or – perhaps of special interest to pediatric and some addiction work – losing teeth. When this is overlooked, it can cause problems with data interpretation (e.g., inflated biomarker levels). There are a number of ways to prevent or detect blood contaminations in oral fluid samples. Prior to sample collection, participants can be prescreened to assess oral health behaviors (e.g., report on blood following brushing or flossing, access to oral healthcare) that may predispose them to blood in their saliva. Once samples have been collected and prior to laboratory analyses, a rating scale can be applied by laboratory technicians to determine if contamination is visible indicating the resulting likelihood of sample reliability. Finally, the comeasurement of transferrin in the samples being quantified will allow for detection of blood contamination [65].

Additionally, the type of participant or animal being studied should be considered by researchers interested in incorporating oral fluid assessments. For many analytes, a small amount of sample can be used for analyses; however, for some individuals it may be difficult to obtain the minimum amount required for analyses. This may be of special consideration for elderly individuals or those who are on certain medications, whereby dehydration or reduced oral fluid production result in minimal amounts of fluid available for collection. In animal models, special consideration for those animals that may require restraint for oral fluid assessments should be taken, as this may affect not only the ability to obtain a sample, but also the resulting analyte levels (e.g., potential elevation of stress hormones due to restraint).

Conclusion & future perspective

After 30 years of progress, the science of ISB has accumulated scientific critical mass. Many different types of biological parameters reflecting various levels of biological analyses can now be measured in oral fluids, opening up vast opportunities for advancement across biological and psychology sciences. Moreover, the integration of biological parameters enables behaviorally oriented studies to be closely aligned with the national research agenda (e.g., National Institute of Mental Health, Research Domain Criteria). The continued growth and diversity of newly available oral fluid measures may foreshadow a period of innovation and discovery. Thus far, salivary bioscience measurement capabilities have advanced to high standards of precision, reproducibility and internal validity. Modern assays are accurate to 1 trillionth of a gram (pg/ml) and are, in some instances (i.e., cytokines) capable of measuring multiple analytes simultaneously from the same sample. As salivary bioscience continues to mature, the advances revealed have potential to drive cross-disciplinary innovation and discovery that has yet to be seen. Over the next 5–10 years, ISB is expected to grow significantly, particularly in the areas of drug monitoring, viral testing, antibody monitoring, field sobriety testing, and direct-to-consumer home testing for illness and disease. The continued advancement of technology used for assessments, investigations into the utility of oral fluid for various biomarkers and growing opportunities for training specific to salivary bioscience is necessary to drive innovation and far-reaching clinical acceptance in the many fields that are a part of ISB.

Disclaimer

The views expressed in this article reflect the results of research conducted by the author, MK Taylor, and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the US Government..

Author contributions

The authors certify that all coauthors have met the criteria for authorship on this paper, including substantial contributions to the conception, basis and writing of this review; drafting and/or revision of this manuscript; final approval of this work for publication; and agreement to the accuracy and integrity of this work.

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Executive summary

Background

- Salivary bioscience has emerged as a modern interdisciplinary field.

Milestones, historical markers & vista points

- Major points are discussed that were paramount to the advancement of salivary bioscience, including studies that established measurement of stress-related and reproductive hormones in saliva, the first commercially available point-of-care salivary immunodiagnostic assays, the link between poor oral health to risk for chronic disease for the first time via the US Surgeon General's report on oral health, characterization of the salivary proteome, salivary testing by commercial entities and COVID-19 testing.
- Advances enable a diverse range of biomarkers to be measured in oral fluids.
- Standards are established for salivary work from collection to analysis.

Interdisciplinary by nature

- Salivary bioscience is most effectively executed by an interdisciplinary team.

High-impact innovation & discoveries across disciplines

- Applications for oral fluid assessments in medicine, biology, oral health, biobehavioral research and clinical work are discussed.
- This includes psychological studies assessing hypothalamic–pituitary–adrenal axis or the sympathetic branch of the autonomic nervous system, advancing key theories and hypotheses; ecological momentary assessment field work; genetic-based research; COVID-19 considerations; chemical exposures; sleep research; clinical applications, including drug monitoring and drug abuse; stress reactivity; behavioral neuroscience; oral health; and animal research and veterinary medicine.

Specialized strategy & tactics

- The value of salivary measures is revealed using specialized analytical tactics and strategies.
- Noninvasive collection of oral fluid allows for multiple collection time points that may span multiple days, and can be included when assessing acute events.
- Advanced analytical techniques (e.g., latent-state trait modeling) are enabled by these multiple assessments.

Special considerations & potential limitations

- Special considerations for oral fluid collection and analyses include sample type (blood vs saliva), sampling location, collection device, timing of sample collection, measurement of flow rate and laboratory considerations.
- Limitations including the potential for blood contamination in oral fluid samples and potential issues with participant/animal collection are discussed.

Future perspective

- Integration of salivary bioscience has advanced multiple subject areas (e.g., psychological science, infectious disease) and there is unrealized potential.
- Some areas of interest that are currently being explored and are expected to grow due to the integration of salivary testing include drug monitoring, viral testing, antibody monitoring, field sobriety testing and direct-to-consumer home testing for illness and disease.

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Papers of special note have been highlighted as: • of interest; •• of considerable interest

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- **The US Surgeon General's first report on oral health identifies poor oral health as a silent epidemic in North America, outlines the links between poor oral health and risk for chronic disease and focuses attention on salivary measures as intrinsically important in their own right – not just as surrogates of their circulating counterparts.**
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