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Salivary Bioscience and the Future of Behavioral Medicine

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Abstract

Behavioral medicine research from across the globe has been catalyzed by the quest to understand the interactions between psychological, social, and physiological factors underlying disparities in human health. A more complete biopsychosocial model increasingly integrates advanced clinical and laboratory assessments of relevant environmental chemicals, biological mediators of inflammation, cardiometabolic and endocrine markers, infectious disease exposure, and genetic polymorphisms determined from saliva specimens. The overarching aims are to identify mechanisms, decode moderating processes that translate adversity into risk, and verify the impact of clinical intervention. This special issue of the *International Journal of Behavioral Medicine* highlights novel contributions of salivary bioscience with emphasis on research utilizing varied research designs (i.e., experimental, longitudinal, dyadic), incorporating a broad array of salivary analytes, and investigating the influence of psychological and social factors on human health.

Keywords Salivary bioscience · Behavioral medicine

Introduction

Over the past few decades, behavioral medicine researchers have been empowered by minimally invasive means of monitoring biological and physiological processes using oral fluids as a biospecimen (see [1] for review). Advances in technology have allowed evaluation of individual differences between, and changes within, individuals' physiological processes in ways not accessible in the past. These tools enable repeated measurement of the physiological components of interacting biobehavioral processes in the laboratory and, more importantly, in the context of everyday life (e.g., home, work, school, play) and among communities in which collecting traditional biospecimens would be prohibitive due to societal or cultural norms. Such designs have aided in understanding complex stress dynamics, behavioral influences on disease processes, the mechanisms of health disparities,

and how to optimize behavioral interventions to benefit health. Across behavioral medicine, applying these new technologies underscores the importance of *context* as a determinant of the expression of biological variables and as a moderator of relationships between biological processes and health-relevant outcomes. Correspondingly, salivary bioscience has ushered in advances not only in measurement but also in conceptual modeling and analytical strategies and tactics required to test those models [2]. The purpose of this special issue of the *International Journal of Behavioral Medicine* is to highlight recent advances and draw attention to the promise that salivary bioscience has to offer the future of behavioral medicine research.

The Evolution of Salivary Bioscience

The origin story of contemporary salivary bioscience can be traced to basic research on oral biology. As reviewed by Granger and Taylor [3], several scientific events contributed to rapid expansion of interest in, and attention to, oral fluids as research and diagnostic specimens. Among the first were studies in the 1980s revealing that hormones (e.g., cortisol, testosterone, estradiol, progesterone) were measurable in oral fluids and that quantitative measures of these protein hormones were strongly associated with their corresponding levels in the blood.

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A second event occurred in the early 1990s in response to the HIV-AIDS pandemic: a commercially available salivary immunodiagnostic assay for infectious disease exposure (Orasure, Bethlehem, PA). A third involved the capacity to measure metabolites of therapeutic medications and drugs of abuse in oral fluids [4]. Finally, the publication of the first US Surgeon General's Report on Oral Health [5] underscored the links between poor oral health and risk for diabetes and cardiovascular disease.

Today, salivary bioscience continues to evolve and has reached new heights. New windows of opportunity have been opened for behavioral medicine research by, for example, the characterization of the salivary proteome [6]; the ability to obtain high-quality and quantity human DNA, yielding genetic polymorphisms and enabling the assessment of epigenetic markers [7]; the capacity to measure the human oral microbiome and metabolome [8]; and the utility of salivary cardiometabolic markers [9, 10]. The integration of contemporary salivary bioscience with behavioral medicine research seems timely, strategic, and low risk with the potential for high reward.

Saliva Secretion, Composition, and Constituents: a Primer

Understanding whether a salivary analyte (your substantive measurement interest) is transported into oral fluid by filtration or passive diffusion, secreted from salivary glands, or released or derived from cells locally in the oral mucosa is key to interpreting individual differences in that measure (e.g., [11]). This necessitates understanding how saliva is secreted into the oral cavity as well as its composition and components. In fact, what most of us believe to be “saliva” is actually a composite of oral fluids secreted from many different glands located in the upper posterior area of oral cavity, lower area of the mouth between the cheek and jaw, and under the tongue (see [12] for review). A small portion of oral fluid (crevicular fluid) comes from serum leakage in the cleft area between each tooth and its surrounding gum tissue. Each secretory gland produces a fluid that differs in volume, composition, and constituents [13, 14].

Oral fluid is water-like in composition and is neutral or only slightly acidic when unstimulated. Serum constituents are transported into saliva either by *filtration* between the tight spaces between cells in the salivary glands or by *diffusion* through those cells' membranes. Other analytes found in oral fluids are synthesized, stored, and released from the granules within the secretory cells of the saliva glands (i.e., enzymes, mucins, cystatins, histatins). Still others are components of humoral (antibodies, complement) immunity or proteins (cytokines) secreted by cells (neutrophils, macrophages, lymphocytes) of the mucosal immune system.

“Scholarship” and Salivary Bioscience

There are specialized issues involved in the measurement, interpretation, and reporting of salivary analytes. Theoretical integration of biological processes with existing behavioral medicine theories is key. Equally important are best practices for sample collection, cold chain management, transport, and storage; the sensitivity of various markers to external and internal factors; and limitations of assay precision and reproducibility (see [15, 16] for a more complete discussion). This specialized knowledge is one reason why investigators from behavioral science backgrounds often choose to work or train in interdisciplinary teams when using salivary bioscience methods. As we considered submissions for this special issue, we identified a handful of themes for consideration by both authors and reviewers in an effort to improve our ability to compare studies and to enhance the reliability and replicability of results across the field:

- *Theoretical/conceptual integration.* Behavioral medicine research has long been distinguished by its guiding use of theory in hypothesis generation, study design, and identification of mechanisms and processes. Objective measures of health and biological processes are critical to the development, refinement, and application of behavioral theories. Principles of high-quality research design should be used when integrating salivary biomarkers. Salivary biomarkers should fit within sound conceptual and theoretical frameworks, including nuanced understanding of the specific biological processes of interest. Reporting should include description of how the biologic process(es) of interest is considered within the conceptual framework, how the salivary marker reflects the biologic process of interest, and how it relates to the health process of focus.
- *Sample collection procedures.* Salivary assays have progressed in their precision, lower limits of detection, and reproducibility to meet rigorous standards of the immunodiagnostic community. Currently, the majority of error variance in salivary analyte data is contributed during sample collection, storage, and transport, and not in the analytical laboratory. Reports need to clearly state the methods of sample acquisition, including the type of collection device and the specific procedures of use. Reports must describe what specific type of oral fluid was obtained (whole saliva, glandular oral fluid, or crevicular fluid). If the salivary analyte of interest is flow-rate dependent (some are particularly sensitive), reports should include the manner in which saliva flow rate was measured and considered. Finally, saliva production should not be stimulated. If stimulation is essential, preliminary or pilot data should show that salivary stimulation did not compromise sample integrity.

- *Sample handling, transport, and storage.* The microbial community of the mouth is extremely diverse. Once specimens are collected, they should be kept cold or frozen, and once frozen, the samples should stay frozen until the day of assay. Refrigeration prevents degradation of some salivary analytes and restricts the activity of proteolytic enzymes and growth of bacteria. For large-scale national surveys, investigators working in remote areas, or patients collecting samples at home, freezing and shipping these frozen samples can be logistically complex and cost prohibitive. Under some circumstances, saliva may not be the biospecimen of choice. Investigators should report the details of cold chain management and track for each specimen the number of freeze-thaw cycles. Cold chain monitoring should document all periods of freeze and thaw from the time the sample is taken through time of laboratory assay.
- *Poor oral health.* To meaningfully index *systemic* biological activity, quantitative estimates of an analyte (e.g., hormone) in saliva must be highly correlated with serum levels. The magnitude of this serum–saliva association depends, in part, on the consistency of the processes used to transport circulating molecules into oral fluids [17]. When the integrity of diffusion or filtration is compromised, the differential concentration gradient will affect transportation from serum to saliva. To illustrate, blood in oral fluid is more prevalent among individuals who suffer from poor oral health (i.e., open sores, periodontal disease, gingivitis, inflammation). Investigators should be aware of, document, and control for (if possible) oral health status [2] and also document when saliva is contaminated with blood [18, 19].
- *Sample collection timing adherence.* Several metrics of interest in salivary bioscience require precise assessment of the time a saliva sample was collected. Salivary bioscience has increasingly come to appreciate the importance of this issue [20]. Investigators should report the methods and tactics they employed to document collection time adherence, and the decision rules they applied to exclude samples from analyses based on non-adherence to the collection protocol. This precision is especially relevant for salivary studies of the cortisol awakening response or diurnal slope of cortisol production, as well as studies using saliva to assess melatonin in protocols assessing Dim Light Melatonin Onset or DLMO [21].
- *Assay procedures.* Descriptions of research methods detailing the determination of salivary analytes should minimally include the following: type of assay, assay manufacturer (if a commercially available assay is used), modifications to the recommended protocol if any, sample test volume in microliters, range of calibrators, lower limit of detection, average inter- and intra-assay precision for samples tested in the study, and percent of samples at

undetectable levels. Riis and colleagues [2] provide recommendations and best practices on censoring and transforming salivary bioscience data.

- *Analytical strategies and tactics.* In addition to this basic reporting, the ability to collect multiple samples over time per participant and assay multiple analytes per sample requires sophisticated analytical strategy. Riis and colleagues [2] provide insight into several of these modeling methods using salivary analytes. Guidance on physiometrics included in this special issue [22] also offers reporting considerations for salivary analyte measurement and data. Appropriate use of and clear reporting on statistical models are necessary to answering complex behavioral medicine questions informed by salivary biomarker data.

IJBM Special Issue in Salivary Bioscience

The articles in this issue of IJBM illustrate the innovative use of salivary bioscience to answer important behavioral medicine questions. These studies utilize a variety of research designs and an array of salivary analytes. With this issue, we highlight the potential for salivary bioscience to move behavioral medicine forward. For instance, social processes can get under the skin to impact biological functions. Hooker and colleagues ([23]; this issue), in a daily diary study, demonstrated that socioeconomic status matters in the day-to-day relationships between social support and cortisol. The ability to collect ecologically valid psychological and salivary measures across the course of several days revealed the dynamics of psychological, social, and biological processes. Highlighting the value of examining biological change under more controlled conditions, Shirotaki and colleagues ([24]; this issue) employed experimental techniques to show that social evaluative threat exerts a negative toll on health. Their focus on the cortisol/dehydroepiandrosterone (DHEA) ratio provides an example of viewing a biological process within a biological context; the anti-glucocorticoid effects of DHEA counters those of cortisol.

Longitudinal investigations provide strong evidence for health impact. Eiden and colleagues ([25]; this issue) lent insight into the potential impact of prenatal tobacco and cannabis exposure and physiological stress responses in later development. The moderating impact of parental factors on cortisol reactivity revealed the complex and dynamic relationships of physiological, behavioral, and environmental factors.

The preponderance of behavioral medicine studies of salivary biomarkers focus on cortisol (see [1]). However, Riis and colleagues ([26]; this issue) and Slavish and colleagues ([27]; this issue) utilized salivary measures of inflammation. The measurement of inflammatory markers in saliva remains nascent, and both articles pointed to the promise and potential

pitfalls in salivary measures of inflammation. Both papers indicated that although salivary measures of inflammatory markers appear to be associated with systemic levels, the degree of concordance and the influencing concomitants are emergent areas of focus. Slavish and colleagues ([27]; this issue) examined the associations between salivary markers of CRP and IL-6 and positive and negative affect over a 2-week period. Basic psychological studies such as this one will better characterize salivary inflammation markers. Riis and colleagues ([26]; this issue) elegantly investigated the relationship of prenatal factors and neuroendocrine-immune dynamics over time. The association between higher prenatal cortisol exposure and stronger cortisol-cytokine relations at age 5 highlights the promise of salivary cytokine measures. Advances in assay technology are improving confidence in salivary inflammation measures, though more work to establish the validity, long-term stability, and optimal measurement strategies is needed.

Behavioral medicine is only beginning to expand its consideration of salivary analytes. This issue aims to bring attention of behavioral medicine researchers to emergent areas. For instance, few behavioral medicine studies have incorporated measurement of salivary estradiol. Rieder, Darabos, and Weierich ([28]; this issue) call on research from neuroscience, clinical psychology, and reproductive medicine to illuminate estradiol's role in psychological distress and its potential influence on somatic symptoms and health behaviors. Advances in salivary measurement of estradiol provide an opportunity for behavioral medicine researchers to understand a variety of health contexts including prevention, adjustment to chronic illness, and symptom management. Martin and Ter-Petrosyan ([29]; this issue) measured salivary testosterone in a sample of female college students to investigate relationships between testosterone and health behavior engagement. Finally, Ali and Nater ([30]; this issue) contributed invited commentary on the use of salivary alpha-amylase (sAA) in behavioral medicine stress research. A reliable marker of autonomic stress activity, sAA, has a growing role in understanding the impact of stress on disease. Measurement of multiple physiological stress markers will provide a more comprehensive picture of the impact of stress and better insight into the differentiation of stress systems (e.g., [31]).

Concluding Comment

The interdisciplinary nature of behavioral medicine has expanded and so too have its basic scientific and clinical practice missions. Its distinguished history in understanding behavioral influences on the onset, prevention, and management of disease and on the promotion of well-being and health points the way toward a future of continued innovation and discovery. Indeed, the stage is set for a new era. The integration of technologically valid and accessible measurement of

biological processes with ecologically valid and meaningful contexts can advance behavioral medicine to new levels. With this objective in sight, this special issue introduces the current state of the art of salivary bioscience and emphasizes its trans-disciplinary contribution to behavioral medicine research.

Compliance with Ethical Standards

Conflict of Interest The authors declare they have no conflict of interest. In the interest of full disclosure, DAG is founder and chief scientific and strategy advisor at Salimetrics LLC and Salivabio LLC. These relationships are managed by the policies of the committees on conflict of interest at the Johns Hopkins University School of Medicine and the University of California at Irvine.

Informed Consent This article does not report any human subjects data and therefore no consent was necessary.

Ethical Approval For this type of paper, formal consent is not required.

Human Subjects This article does not contain any studies with human participants performed by any of the authors.

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