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Salivary Bioscience

Foundations of Interdisciplinary Saliva Research and Applications

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Preface

Thousands of investigators worldwide are actively engaged in basic, applied, and clinical research involving salivary bioscience. Our literature search reveals that across the past two decades the number of empirical papers published annually has increased substantially; in 2019 more than 1500 empirical salivary bioscience papers were published. Investigators engaged in salivary bioscience span many academic disciplines including medicine, public health, psychology, sociology, education, neuroscience, biological science, animal behavior and welfare, infectious disease epidemiology, social neuroscience of human–animal interaction, drugs/drug abuse, social networks, nursing, psychoneuroendocrinology, anthropology, cognitive science, bioengineering, dentistry, oncology, oral health, and pediatrics.

To date, the foundational information in salivary bioscience (i.e., 25 years of literature) is not easy or efficient to find. It is scattered across many different journals, over at least two decades, and some of the early work has been subsequently shown to be in error. This makes it challenging for new investigators interested in the topic to find the "right" information on their own. Unfortunately, and to the best of our knowledge, there is no definitive state-of-the-art guide to interdisciplinary salivary bioscience research. Over the years there have been edited volumes from the proceedings of highly specific conferences. Understandably, the nature of these presentations is highly technical and narrow in scope, and the content chapters in those texts are written for an audience of highly trained experts. By contrast, this edited volume is written by leaders in multiple fields and fulfills a demand for a *broad understanding of salivary bioscience* across a range of disciplines.

Douglas A. Granger

Acknowledgements

The history of the emergence of interdisciplinary salivary bioscience has been influenced by many mentors, advisors, early adopters and visionaries over many years. Here we call attention to some of these key individuals for the significant roles they played in the development of the foundation of knowledge that scripted the "big picture"—Daniel Malamud, Lawrence Tabak, Harold Slavkin, John R. Weisz, James T. McCracken, John L. Fahey, Barbara Henker, Herbert Weiner, Dirk Hellhammer, Ben Weigand, Margaret Kemeny, Lynn Kozlowski, Elizabeth Susman, Alan Booth, James Dabbs, Jr., Peter Ellison, Lynn Vernon-Feagans, Ann Crouter, Megan Gunnar, Dante Cicchetti, Dan Leri, Clancy Blair, Martha N. Hill, Gayle Page, Deborah Gross, Robert Blum, Janet Dipietro, Tina Chang, Keith Crnic, Cary Savage, Karen Rook, Dele Ogunseitan, and Nancy Guerra. We also call attention to some of the many technical and operational experts who have made significant contributions—Najib Aziz, Eve Schwartz, Mary Curran, Skip Nelson, Laurie O'Brien, Tracy Hand, Jon Peterson, Rebecca Zavacky, Jessica Acevedo, Lillian Buitenhuys, Kelly Henning, Greg Reinhard, John Stebbins, Kaitlin Smith, Hillary Piccerillo, and Anthony Tette.

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Editors and Section Editors

About the Editors

Douglas A. Granger Ph.D., is engaged in multi-institution research focused on the discovery, measurement, and application of analytes (e.g., enzymes, hormones, antibodies, chemicals, elements, and cytokines) in saliva. He is a Chancellor's Professor and Director of the Institute for Interdisciplinary salivary bioscience research (IISBR) at the University of California at Irvine and holds adjunct faculty positions at Johns Hopkins University. His studies have been instrumental in the conceptualization and analysis of biosocial relationships involving child well-being, parent–child and family relationships, as well as how these biosocial links moderate and mediate the effects of adversity and stress on health and development.

Marcus K. Taylor Ph.D., is an Institute for Interdisciplinary salivary bioscience research (IISBR) Faculty Affilitate at the University of California at Irvine and a Fellow of the American College of Sports Medicine. He previously held faculty positions at Duke University and San Diego State University. His program of research focuses on stress physiology in extreme environments, blast exposure, and gene–environment interactions. His recent work appears in *Journal of Special Operations Medicine, Psychoneuroendocrinology, and Psychiatry Research*.

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Elizabeth A. Thomas Ph.D., is an Adjunct Associate Professor in the Department of Neuroscience at the Scripps Research Institute and researcher in the Department of Epidemiology and IISBR faculty affiliate at the University of California at Irvine. Her research program seeks to investigate epigenetic, biochemical, and translational biomarkers in clinical populations with neurodegenerative and/or neuropsychiatric

disorders. Overall, the goals of these discovery and translational research studies are to improve the diagnosis, prevention, and treatment of patients with these devastating disorders.

Jenna L. Riis Ph.D., is an Assistant Professor of Psychological Science at the University of California at Irvine and member of the Institute for Interdisciplinary Salivary Bioscience Research. Her program of research focuses on the etiology of health disparities and the processes by which early-life adversity affects child development and lifelong health. Dr. Riis' research examines the biopsychosocial mechanisms underlying health and development from an integrated, multisystem perspective and includes studies of novel salivary markers of the immune, metabolic, and purinergic systems.

Kate R. Kuhlman Ph.D., conducts research on the biobehavioral processes through which adversity in childhood leads to health disparities across the life span, with a particular interest in stress-related disorders such as depression. She is currently an Assistant Professor in the Department of Psychological Science and the Institute for Interdisciplinary Salivary Bioscience at the University of California Irvine, as well as a member of the faculty in the UCLA Cousins Center for Psychoneuroimmunology.

Part I
What Is Salivary Bioscience, Why Is It Important, and How Do We Study It?

Douglas A. Granger and Marcus K. Taylor

Chapter 1
Foundations of Interdisciplinary Salivary Bioscience: An Introduction

Douglas A. Granger and Marcus K. Taylor

History reveals that advances in our scientific understanding often accelerate after technological innovations improve upon our ability to observe and measure phenomenon more precisely (e.g., Kuhn, 1962). Also, more often than not, big leaps in knowledge are the result of the collective effort of teams of collaborating investigators rather than by individual scientists working in relative isolation independently. At least part of the argument in favor of team science is that the nature of most key phenomenon under study involves factors operating at multiple levels of analysis. Indeed, contemporary theorists assume that complex phenomenon, such as human development, disease, poverty, and public health, are determined by a confluence of effects involving interacting intrinsic individual differences, behavior, biological, and contextual factors. A major advantage of team science is that individual team members represent a deeper level of knowledge in a particular field or level of analysis than is efficient for any particular investigator to achieve and maintain. That, in theory, enables problems to be approached from dynamic inter- and trans-disciplinary

In the interest of full disclosure, Douglas A. Granger is the founder and chief scientific and strategy advisor of Salimetrics LLC and Salivabio LLC (Carlsbad, CA) and the nature of these relationships is managed by the policies of the committees on conflict of interest at Johns Hopkins University School of Medicine and the University of California at Irvine.

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perspectives with the intellectual capacity of the scientific collective being greater than the sum of the individual's knowledge (Stokols, Hall, Taylor, & Moser, 2008).

As a partial consequence of these macro-level scientific trends, there has been widespread integration of biological processes into contemporary conceptual and measurement models in traditionally nonbiologically focused areas of inquiry. The last 2–3 cohorts of PhD trainees would most likely accept this as the norm, given the adoption of this approach prior to the period in which they have been trained. Yet, on the scientific evolutionary time scale, this has been just recently realized. Until advances in technology enabled the minimally invasive measurement of biological variables this was not commonplace. What is interesting is that before 1950, discoveries enabled the minimally invasive measurement of biomarkers and analytes in oral fluids. Yet, the impact of these advances was largely limited to sub-specialties within oral biology generally, and clinical dental research specifically, until perhaps the 1980s.

In the 1980s, observations by investigators interested in the psychobiology of stress, such as Dirk Hellhammer and his students at Trier University, explored the possibility that circulating levels of cortisol could be accurately estimated using salivary measurements. Advances like these sharply focused empirical attention of many investigators and the results of that effort ushered in more than 4 decades of research involving the integration of salivary analytes in behavioral and social sciences. In parallel, within the field of infectious disease surveillance, the early stages of the HIV epidemic in the 1980–1990s focused empirical and commercialization (e.g., Saliva Diagnostics Systems, Orasure) efforts to enable a minimally invasive means of screening HIV antibody sero status (e.g., without risk of accidental needle stick). Pioneers of this early era of salivary bioscience included dental researchers such as Irwin Mandel, Lawrence Tabak, and Daniel Malamud. The need for a safe and effective screening method in the context of this widespread epidemic began the effort to chart the potential of oral fluid as an alternative to traditional diagnostic specimens. As a result of their individual scientific achievements and the efforts of their extensive network of international colleagues, a series of scientific meetings was organized and sponsored by the New York Academy of Sciences. The proceedings of these conferences resulted in at least two seminal publications describing the current state of knowledge. The first was a 342-page edited volume titled "*Saliva as a Diagnostic Fluid*" (Malamud & Tabak, 1994), which was followed up years later by the 514-page edited volume titled "*Oral-based Diagnostics*" (Malamud & Niebala, 2007). Concurrent efforts in the 1980–1990s by Peter Ellison and James Dabbs expanded the range of small molecule salivary measurements to include progesterone, estrogen, and testosterone—adding new dimensions to the study of hormones and human behavior, health, and development. The widespread enthusiasm surrounding the potential of oral fluids as a diagnostic specimen lead to several new research initiatives at the US National Institute for Craniofacial and Dental Research (NICDR). Noteworthy is the fact that this effort, in addition to funding an extensive portfolio of individual investigator initiated projects, supported a groundbreaking multisite collaborative to characterize the Human Salivary Proteome. The

library produced by the project opened the windows of opportunity widely—hundreds if not thousands of analytes were identified in oral fluids (e.g., Yan et al., 2009). Among the many indications of the high impact of this effort is that salivary bioscience was included by Harold Slavkin as one of the key pillars in the first US Surgeon General's report on oral health (US Department of Health and Human Services, 2000). Moreover, as a consequence of the human genome project, a strong pulse of research activity demonstrated the capability to extract high quality and quantity DNA from oral fluids (e.g., Nemoda et al., 2011). Another major research emphasis was focused on the assessment of drugs of abuse and/or their metabolites in oral fluids. These capabilities, in particular, lead to the realization of niche commercial enterprises with specialized applications in consumer-based testing, health care, insurance, and law enforcement.

Quite surprisingly, given the obvious advantages, the scientific and commercial enthusiasm for *saliva as the diagnostic fluid of the future*, with a few notable exceptions (i.e., DNA, HIV screening, and some drug testing) has waxed and waned over the years. Many saliva-based opportunities have attracted considerable public interest and also substantial financial investment, but more often than not these ventures have failed to deliver commercially viable devices or clinically meaningful saliva-based assessments. There are of course isolated examples of success (e.g., Orasure, Salimetrics, 23-and-me, Tecan-IBL), but generally speaking, saliva as a *diagnostic* specimen has yet to live up to the initial enthusiasm expressed by the clinical and business communities about its potential (see for instance Malamud & Tabak, 1994). Explanations range widely from legal, regulatory, and economic barriers to entry, to concerns about precision and reproducibility, to the interpretation of measurements made from oral fluid samples.

By stark contrast, in the last two decades, this effort has led to a renaissance in the traditionally behaviorally oriented basic and applied sciences. In fact, the number of peer-reviewed scientific publications involving salivary analytes has increased from a handful to multiple thousands annually within the past 20 years. For instance, Fig. 1.1 displays the results of a pubmed.gov search using only the key words "salivary cortisol"—revealing the number of annual publications increased from a handful in 1985 to more than 400 in 2019. Similar "accelerating trends" are evident in the publication rates for many other salivary analytes (e.g., sIgA, testosterone, C-reactive protein, and alpha-amylase). The scientific research often involves a focus on testing innovative theoretical models of individual differences in health, behavior, and cognition as a function of multilevel biosocial processes in the context of everyday life.

The ease of use and minimally invasive nature of saliva collection is especially valued in this endeavor because complex multilevel models of individual difference can be studied in the laboratory, in quasi-naturalistic settings, or in response to the trials and tribulations of people's (and animals') everyday social worlds. Also, multiple time point sampling can be undertaken, in many circumstances, without adding significant participant burden or interrupting the natural flow of activity. Significant progress has been made, especially during the 15-year period between 1995 and 2010, to develop and refine saliva collection and measurement methods,

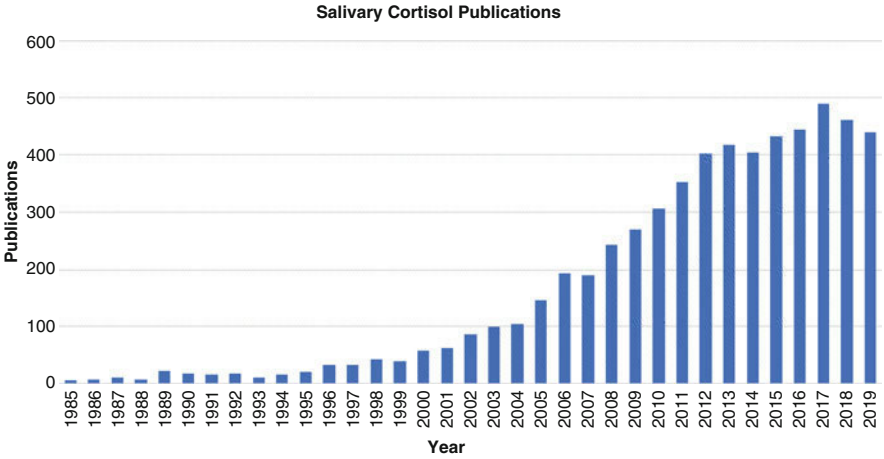

Fig. 1.1 An illustration of the increasing number of salivary bioscience publications. The search period was from 1985 to 2019 (the last year of complete data as of the publication date). The search engine was PubMed, and keywords for this illustrative search were "salivary cortisol." As can be seen in these historical data, the level of research activity in salivary bioscience has substantially increased over the last 20 years. Similar findings emerge when searches are conducted with many other salivary analytes

many of which are now commercially viable. It is reasonable to conclude that since mid-2000, the scientific community has had a very advanced understanding of how to collect, handle, transport, store, assay, and interpret saliva-derived biological data.

Simple database searches (e.g., PubMed, Psychinfo, Scopus, and Web of Science) are sufficient to reveal that not only is the number of publications incorporating salivary measures increasing, the number of scientific fields in which salivary analytes has been employed is also expanding. The scan of PubMed search results between 1980 and 2019 for keywords "salivary cortisol," noted above, for example, yields more than 5500 total publications. Across the years, the range of scientific fields (represented by the journal titles) is also increasing. In 2019, publications involving salivary bioscience appeared in journals linked to dentistry, psychiatry, psychology, sports science, endocrinology, sleep, neuroscience, nursing, circadian biology, obesity, anthropology, public health, child development, psychosomatic medicine, and aging. The number of different salivary analytes involved in this research effort is also expanding, and now includes, for instance, measures of hormones, cytokines, immunoglobulins, enzymes, DNA, environmental chemicals, and elements and metals.

The significance, importance, and impact of this scientific integration was marked on a scientific evolutionary time line in 2010, when Johns Hopkins University created the Center for Interdisciplinary Salivary Bioscience Research, then again in 2016 when the University of California executed a campus wide strategic initiative to create the Institute for Interdisciplinary Salivary Bioscience Research. In parallel, on the international scientific stage, similar events mark this developmental

milestone with the creation of salivary bioscience laboratories and centers at the University of New South Wales in Sydney Australia, Angelia Ruskin University in Cambridge United Kingdom, Charite University in Berlin Germany, and the Institute for Experimental Medicine of the Hungarian Academy of Science in Budapest Hungary. Noteworthy is the absence of the term "diagnostics" and the inclusion of the terms "interdisciplinary" and "bioscience" in the labeling of many of these academic units.

A core assumption in the "saliva diagnostic era" was that a salivary measure is primarily of interest because of the extent to which that measurement is highly associated with the corresponding measurement in the general circulation. The core idea has been *saliva as a surrogate of blood*. Today, this possibility reflects only a subcomponent of a much broader set of assumptions. That is, consistent with the hypothesis forwarded by Harold Slavkin, *Salivary Bioscience* assumes that the oral cavity serves as a window to the body, measurements made in oral fluids (even if they are not directly correlated with parallel measures in circulation) have the potential to be important indicators of health, disease, and physiological states in their own right, and in addition to the measurements made in traditional biological specimens (US Department of Health and Human Services, 2000). On the other hand, it has also been realized that not every measure derived from a blood sample can be determined in an oral fluid sample. Therefore, saliva is unlikely to be a replacement for traditional diagnostic specimens in many circumstances. For instance, in a clinical setting it is often the case that a panel of measurements is determined from a blood sample. If only a subset of those measures is possible in an oral fluid sample, then a blood draw is necessary and the determinations from an oral fluid sample are redundant and add little value.

The contemporary interdisciplinary perspective—*salivary bioscience* in contrast to *saliva diagnostics*—allows investigators to consider that the conceptual, methodological, and empirical advances to date will enable us to determine for whom, under which circumstances, which measurements made in oral fluid might add value. Beyond the minimally invasive nature of sample collection is the opportunity saliva affords for investigators to collect multiple measurements from individuals and explore intraindividual variation. Multiple sampling time points, without adding participant burden, and multiple measurements per time point affords researchers and clinicians many advantages. For instance, multiple time point salivary samples enabling intraindividual assessments of therapeutic drug metabolites might provide clinicians higher resolution in titrating drug dosage to the individual, and might increase the probability of detecting exposure to an environmental contaminant with a short half-life. Furthermore, multiple measurements enable aggregation that may improve the reliability of the estimates of a highly variable analytes concentration, and multiple measures across the day enables estimation of an analytes diurnal pattern of production. Not surprisingly, these advantages of saliva as a research specimen have somewhat outpaced our statistical strategies and tactics—raising new challenges and opportunities.

As our basic knowledge has developed so too have measurement tactics been influenced by changing technologies. The first generation of salivary measurements

were made possible by in-house research use only modifications of radioimmunoassays designed for use with serum/plasma. The modifications were focused on minimizing matrix effects and improving upon the lower limits of sensitivity. These assays were commonplace in research prior to the late 1990s. The next generation of measures were enzyme-based immunoassays specifically designed for use with saliva. These assays are largely still in use and focused on minimizing sample test volumes, optimizing precision, and reducing lower limits of sensitivity to the pg/mL range. The most recent applications of these tactics involve multiplex-coated bead or plate technologies (i.e., Luminex and Mesoscale Discovery). The third-generation measurement strategies involve the use of a variety of point-of-care lateral flow, layered paper, or microfluidic technologies paired in some circumstances with mobile phone applications. POC applications have been proven technically feasible, and qualitative assays have performed well. On the other hand, at the time of this writing, quantitative measurements in saliva using POC technologies struggle with reliability and precision below the low ng/mL range.

The purpose of this edited volume is to serve as a foundational reference text in salivary research methods, data collection, analysis, and interpretation; as well as applications to medicine, surveillance, and monitoring. More specifically, to highlight, document, and benchmark the current state of the art and breath of salivary bioscience from an interdisciplinary perspective. To this end, the editorial team has tasked multiple writing teams to summarize progress in the application of salivary bioscience. Chapters summarize the integration of salivary bioscience in the context of research on neuroscience, stress, genetics, microbiome, immunity, circadian biology, pain, infectious disease epidemiology, behavioral medicine, psychiatry, oncology, periodontal medicine, pain, environmental exposure, drugs of abuse and therapeutic drug monitoring, social neuroscience of human–animal interaction, animal well-being, precision medicine, health policy, aging, and military, and space research. The charge to the writing teams was to summarize progress, to speak to the history, the promises and the prospects, and estimate the "impact" salivary bioscience has on these specific fields, but also identifying the problems and the pitfalls with an eye on the gaps in knowledge, and also best practices and worthwhile future directions. As a collective, the editors and authors anticipate this volume will be particularly inspiring to those scientists, practitioners, and students who wish to make significant contributions to the evolution and eventual maturity of this exciting emerging field.

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Chapter 2
Salivary Gland Anatomy and Physiology

Lisa M. Hernández and Marcus K. Taylor

Abstract In mammals, saliva is a mildly acidic secretion made mostly of water (99.5–99.8%). In a healthy state, humans produce between 500 mL and 1.5 L of saliva per day. Saliva has numerous functions including lubrication, digestion, and immunity. Salivary glands are classified as exocrine, and as such, they produce secretions (i.e., saliva) onto an epithelial surface via a system of ducts. Saliva secretion and production are mediated by the autonomic nervous system (ANS) and thus; salivary glands have both parasympathetic and sympathetic innervation.

Within the oral cavity, there are three major salivary glands; parotid, submandibular, and sublingual, as well as hundreds of minor glands. These glands produce serous, mucous, or seromucous secretions that contain proteins and compounds, which are significant to salivary bioscience studies. Saliva composition depends upon health status and overall physiologic need.

This chapter will delve further into the macro- and microanatomy of the normal salivary gland and will detail the physiologic and neural regulation of saliva production, composition, and secretion.

Keywords Oral cavity · Duct · Submandibular · Parotid · Sublingual

2.1 Basic Anatomy of the Oral Cavity

As the entrance to the digestive system, the oral cavity senses, mechanically processes, lubricates, and initiates the digestion of food (Martini, Timmons, & Tallitsch, 2009). The mouth is lined with epithelial cells and is structurally supported by fat (buccal fat pads) and muscle (buccinator muscles) in the cheeks. The teeth are

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anchored to the maxilla (upper jaw) and mandible (lower jaw) and surrounded by gum tissue. The space where a tooth meets the gum tissue is known as the gingival sulcus where gingival crevicular fluid (GCF) is found. The tongue muscle provides mechanical processing and manipulation of food for chewing and swallowing. It also plays a role in sensory analysis (i.e., temperature and taste) and produces secretions for digestion (i.e., mucins and lipase).

2.2 Salivary Gland Anatomy

Salivary glands are classified as exocrine glands just like sweat, mammary, and sebaceous glands. By definition, they produce and secrete substances via a duct onto an epithelial surface (e.g., the skin or oral cavity). All exocrine glands are categorized into three types, based on the type of secretions that they deliver; (1) Serous glands produce a watery liquid, which usually contains enzymes; (2) Mucous glands yield mucins, which combine with water to form mucus; and (3) Seromucous (mixed) exocrine glands yield both serous and mucous secretions. Additionally, salivary glands are considered merocrine glands as they secrete their products via exocytosis.

Anchored deep to epithelial lining of the mouth, salivary glands are histologically classified according to their structure and secretion, but the basic components are the acinar/alveolar cells, a duct system, and myoepithelial cells. Acinar cells form clusters called acini, which act as the secretory unit of the gland. The epithelium-derived duct system delivers products (Anatomy & Physiology, 2018) to the oral cavity. The length and diameter of the duct system depends upon the type of gland and secretion that is produced (Pedersen, Sørensen, Proctor, Carpenter, & Ekström, 2018). Saliva is first secreted by the acini and thus, the type of acinar cell of the gland dictates the type of secretion to be produced (e.g., serous, mucus, or mixed secretion). Even if a salivary gland has a combination of acinar cells, the individual gland will still predominantly produce serous or mucous secretions.

The fluid that initially constitutes saliva is isotonic, but as it reaches the duct system, it eventually becomes hypotonic (a liquid with more water and less solute than that of blood serum). The tonicity of saliva can be indicative of a basal (unstimulated, more hypotonic) or stimulated state (less hypotonic). The duct system is composed of various cell types; intercalated, striated, and excretory duct cells. Intercalated cells make up the first segment of a duct. The second and third duct portions are made up of striated and excretory cells, respectively. The composition of saliva is altered in these last duct sections where striated cells regulate electrolytes by resorbing sodium. This sodium resorption continues in the excretory duct cells, which also secrete potassium. Finally, the saliva reaches the oral cavity with the help of myoepithelial cells. Located at the base of the acini, and sometimes the intercalated duct cells, myoepithelial cells contract to facilitate salivary secretion. The contraction/relaxation of myoepithelial cells is regulated by the sympathetic or parasympathetic nervous systems, which act upon brain salivary centers (Garrett,

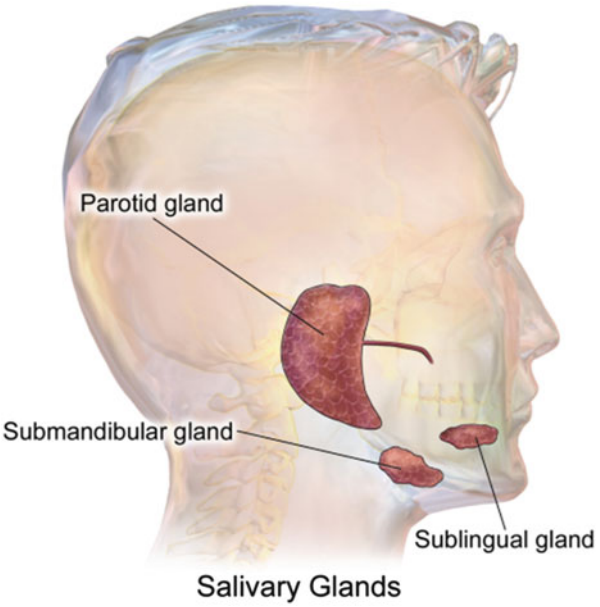

Fig. 2.1 The three major human salivary glands. From https://en.wikipedia.org/wiki/Salivary_gland

1987). Although they are contractile in nature, the myoepithelial cells are not necessary for saliva secretion (Pedersen et al., 2018).

There are three major pairs of salivary glands; the submandibular, parotid, and sublingual glands (Fig. 2.1; Blausen.com staff, 2014). These are classified as major glands based on their anatomical size, and they all have long, branched duct systems as describe above. However, the ducts of the sublingual glands lack striated cells, which means that they resorb sodium to a lesser extent than the other glands. While the major glands produce greater quantities of saliva, they do not necessarily add more to the overall quality of saliva. In fact, major salivary glands contribute the most to volume and electrolyte content, but little to other significant proteins of interest. The submandibular glands produce about 60% of total unstimulated saliva. Inferred by their name, they are located on the floor of the mouth, medial to the lower jaw (mandible). As a mixed gland, it produces a viscous fluid, rich in enzymes and mucins. Mucins combine with water to form mucus, which protects the epithelial lining of the oral cavity by coating food as it makes its way to the esophagus. The parotid glands are the largest glands, but they produce only about 20–25% of total unstimulated saliva. These glands are located just inferior to the cheekbones (zygomatic arches) and are anterior to the ears. Saliva from these glands is serous and abundant in enzymes (e.g., amylase). The sublingual glands are located under the tongue (Fig. 2.1; Blausen.com staff, 2014) and primarily produce viscous saliva that is rich with mucins. In combination with a multitude of other minor salivary glands, they contribute to the remaining 5–10% of total unstimulated saliva. The percent contribution of each gland toward saliva production changes when saliva flow rate is highly stimulated. For instance, the parotid glands increase their yield to account for

50% of total stimulated saliva volume versus 25% under resting conditions (Edgar, 1990).

As previously mentioned, minor salivary glands are only classified as such because of their smaller size, not because of their lesser significance. Minor glands can be found in the lips, cheeks, palate, behind the molars, and on the tongue (Roth & Calmes, 1981). It is estimated that there are 600–1000 minor glands and some are the primary producers of protective components like antibacterial and antimicrobial agents. Like sublingual glands, they lack striated duct cells and they supply a majority of the blood group substances (Edgar, O'Mullane, & Dawes, 2004) found in saliva, such as C-reactive protein (CRP) and some immunoglobulins. Most minor glands produce mucin-rich secretions except for the lingual glands, which generate watery saliva with ample amounts of lipase (Pedersen et al., 2018). Altogether, minor glands produce about 8% of total unstimulated saliva.

2.3 Saliva Composition

Whole saliva, also known as mixed saliva, is a combination of unstimulated and stimulated saliva, microorganisms, gingival crevicular fluid, food debris, and medication metabolites, if any (Fig. 2.2). Habits (e.g., oral hygiene), behavior (e.g., physical activity), and nutritional intake drive the relative contributions of these

Fig. 2.2 Major components of whole saliva

components. Thus, the composition of whole saliva is dynamic and influenced by an exact combination of stimuli. The electrolyte and protein concentration of whole saliva are regulated by circadian rhythms and salivary protein values generally peak in the late afternoon (Rudney, 1995). Electrolyte concentration is largely dependent upon saliva flow rate, which is influenced by health status (e.g., hydration) and overall physiologic needs. In the long-term, whole saliva composition is stable, however; short-term changes in proteins can occur due to daily emotions (Jemmott et al., 1983), respiratory infections (Cockle & Harkness, 1983), inflammation (Henskens, Veerman, Mantel, Van der Velden, & Nieuw Amerongen, 1994), and reproductive status (Cockle & Harkness, 1983; Tenovuo, Laine, Söderling, & Irjala, 1981; Widerström & Bratthall, 1984). These temporary changes may be limited by genetic factors (Rudney, 1995). Systemic disease such as metabolic or immunologic disease also impact saliva protein composition over time. It is therefore important to record an individual's initial health status and then any significant changes thereafter.

Unstimulated and stimulated saliva are the two basic components of whole saliva. Unstimulated saliva is the basal level of saliva production as opposed to stimulated saliva, which is produced in response to chewing (mastication). The submandibular glands are the primary generators of unstimulated saliva. During sleep, this basal production is almost absent. Most stimulated saliva comes from the parotid glands. The sublingual and minor glands contribute equally to both unstimulated and stimulated saliva production. Unstimulated saliva is very hypotonic and has a pH that is neutral or slightly acidic. Stimulated saliva is less hypotonic and has an alkaline pH.

The protein concentration of saliva is inversely proportional to the flow; if there is a high rate of flow, there is less time for the acinar and duct cells to modify saliva and protein concentration is lower. At highest flow rates, saliva is the most isotonic to plasma. Conversely, if flow is at a low rate, protein concentration is increased and saliva is more hypotonic than plasma. The protein content of saliva is highly specific to each person as it is influenced by the individual's genetics, environment, and habits (Rudney, 1995). Most salivary proteins originate solely from the salivary glandular cells and not the blood. Salivary proteins represent only about 10% of the 2500 proteins found in the whole saliva. The remaining 90% is from microorganisms and epithelial cells that are shed from the lining of the mouth (Ekström, Khosravani, Castagnola, & Messana, 2011).

Another innate component of whole saliva is gingival crevicular fluid (GCF), which is found in the gingival sulcus (the space between a tooth and gum tissue). It has a varied composition that is similar to whole saliva, but is produced only in small amounts. It is thought that the main role of GCF is to help to clear food debris and impart antimicrobial/immune protection. Alternatively, GCF has been linked to inflammatory processes, which cause an increase in vessel permeability. Cytokines such as IL-1β, TNF-α, and immunoglobulins G and M, can be found in GCF (Gupta, 2012). Similar to unstimulated saliva, GCF production is regulated by a circadian rhythm with an increase during the typical waking hours and a decline in the late evening. Although it is quite stable during waking hours (Suppipat, Johanasen, &

Gjermo, 1977), GCF production is higher after periodontal interventions, while eating (chewing), if a person smokes, and during hormonal fluctuations in females (e.g., menstruation, oral contraceptives, and pregnancy).

2.4 Functions of Saliva

The constituents of saliva (Fig. 2.3) help to maintain oral health and also facilitate systemic health. Saliva contains electrolytes, immunoglobulins, proteins, enzymes, mucins, and nitrogen products. These entities are multifunctional and work in concert to perform the primary functions of saliva, which are: (1) immunity and antibacterial activity, (2) buffering action, (3) lubrication and tissue protection, (4) taste and predigestion, and (5) tooth integrity. As a primary gateway to the external environment, the oral cavity bears a strong capacity for protection and immune function. Secretory immunoglobulin A (sIgA), the largest immunologic component of saliva, is produced in connective tissue and translocated through the duct cells of major and minor salivary glands (Humphrey & Williamson, 2001). Antibacterial activities are provided by immunoglobulins, proteins, and enzymes. Glycoproteins (proteins attached to oligosaccharide chains) and mucins help to rid the mouth of microorganisms and reduce dental plaque. The pH of saliva in a healthy state ranges from 6.6 to 7.6 (Choi, Lyons, Kieser, & Waddell, 2017) and its buffering capacity is imparted by bicarbonates, phosphates, and urea. Saliva pH is influenced by consumption of sugary or acidic foods (e.g., cherries) and drinks (e.g., soft

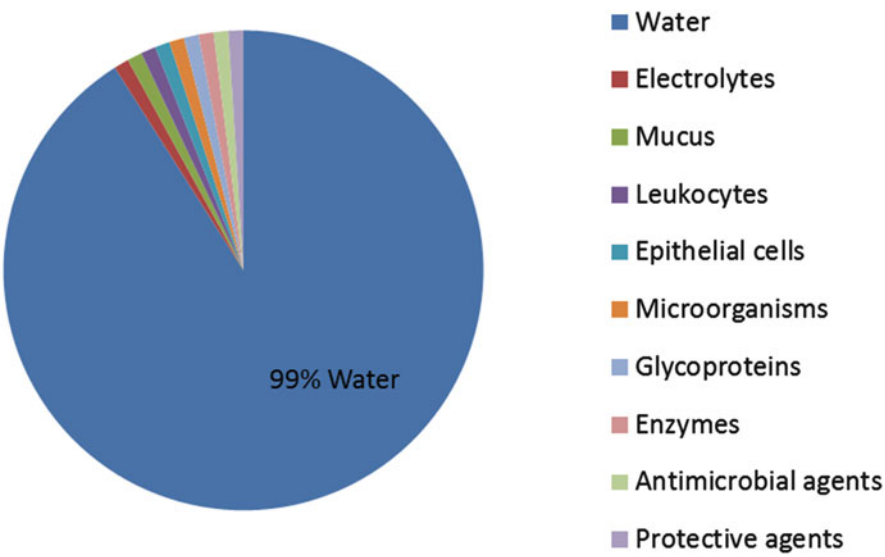

Fig. 2.3 Minor components of whole saliva

drinks), which can temporarily reduce the pH to about 5.5. Mucins help to form mucus, which provides a physical barrier to protect tissues, to soften food for chewing and swallowing, and to facilitate speech. Another major saliva substance is epidermal growth factor, which promotes healing by stimulating DNA synthesis and cell growth/differentiation. Enzymes, such as salivary alpha amylase and lingual lipase, initiate the breakdown of carbohydrates and fats, respectively. Finally, one of the most important functions of saliva is to support tooth integrity. It has been written that "Saliva is to tooth enamel what blood is to the cells of the body." (Moss, 1995). Together, calcium, phosphate, and other proteins combine to form an "antisolubility factor," which modulates the formation of tooth enamel (Humphrey & Williamson, 2001).

2.5 Salivary Flow

Unstimulated saliva production is mediated by the parasympathetic nervous system, which is colloquially known as the "rest and digest" portion of the autonomic nervous system (ANS). Saliva production or volume can also be influenced by environmental and pharmacologic factors, however; the average amount of saliva produced by a healthy adult is estimated to be about 1–1.5 liters/day. Salivary flow is unique to an individual and their responses to internal and/ or external stimuli, but the general range for unstimulated flow rate is about 0.3–0.4 mL/min. Stimulated saliva is the major contributor to changes in waking saliva production. For example, the flow rate in response to food can increase up to a maximum of 7 mL/min (Humphrey & Williamson, 2001). As previously mentioned, unstimulated salivary flow is almost completely absent during sleep. Salivary flow is an important consideration in both clinical and research contexts. Since saliva affords a great deal of immune function and protection, low flow, or hypofunction, can be detrimental. It is important to identify low saliva production, or hyposalivation, under stimulated circumstances, which is defined as a rate <0.1 mL/min. Gland hypofunction can result in an increase in cavities, soft tissue ulcerations, infections, and altered taste (dysguesia) as well as reduced healing from aesthetic dental surgeries and the loss of prosthetic dental restorations (Moss, 1995). Overall, salivary gland function has been reported to be quite robust to the aging process (Pedersen et al., 2018). But, illness, disease, prescription medications (Humphrey & Williamson, 2001; Rudney, 1995), chemotherapy, and radiation (to the head and neck), can cause hypofunction. The anticholinergic side effects of antihistamines or antidepressants may reduce saliva flow and cause xerostomia (dry mouth). Any medications that act upon the beta-adrenergic receptors (e.g., asthma or heart medications) may affect acinar cell protein production. Some antipsychotics, blood pressure, and Parkinson's disease medications may also act on these receptors, resulting in lower flow. Furthermore, hydration status will affect saliva secretion and it has been demonstrated that flow is reduced following restriction of liquid and food (Pedersen et al., 2018). With respect to research, recording hydration status, stress level, presence of respiratory infection, signs of inflammation, and hormone changes (e.g., ovulation and pregnancy) is

prudent since it has been shown that these factors can profoundly affect short-term saliva composition (Rudney, 1995).

Salivary flow is not uniform throughout the oral cavity and there are specific intraoral areas known as the "salivary highways and byways" (Moss, 1995) where flow is either larger or smaller. For example, areas of the lower mouth produce a high volume of saliva while the upper front of the mouth produces very little. Intraoral flow influences the composition of whole saliva as well as composition within different areas of the mouth. This is especially important when instructing a patient or study participant on saliva collection. The analyte(s) being detected will dictate the ideal intraoral area to be sampled (e.g., under the tongue, in the cheek pocket, etc.). Finally, salivary flow is known to change predictably throughout the day (day versus night) and also between seasons (e.g., summer versus winter). Clock genes, which are implicated in circadian rhythm function, have been identified within the salivary glands of mice (Zheng, Seon, McHugh, Papagerakis, & Papagerakis, 2012) and this suggests that flow is regulated by circadian rhythms. To date, the protein expression and characterization of the periodicity of clock genes in human salivary glands have not been reported.

2.6 Neural Regulation of Salivary Glands

Salivary glands are primarily controlled by salivatory nuclei, which are called the "salivary centers." This cluster of nuclei is located in the brainstem (medulla); specifically, in the dorsal pons. Salivary glands also receive input from other brain centers and are influenced by gastrointestinal hormones (Pedersen et al., 2018). The superior salivatory nucleus innervates the submandibular and the sublingual glands. The inferior salivatory nucleus innervates the parotid gland. Both nuclei are components of the main cranial nerves; the superior salivatory nucleus is part of the facial nerve (cranial nerve VII) and the inferior salivatory nucleus belongs to the glossopharyngeal nerve (cranial nerve IX). These nuclei confer parasympathetic input to the glands to produce vasodilation and saliva secretion. In a normal state, there are different types of sensory stimuli for secretion: (1) mechanical, (2) gustatory, and (3) olfactory (Humphrey & Williamson, 2001). In altered states, secretion can be stimulated by pain and pharmacological agents. Other conditions such as depression, fatigue, and fear, can reduce saliva flow (Feher, 2017). In these instances, the common misconception is that salivary flow is reduced by sympathetic inhibitory fibers. In fact, flow is decreased due to "supranuclear control," the influence of higher brain regions like the hypothalamus. Nevertheless, knowledge about the neural regulation of salivary glands is mostly derived from animal studies and the precise connections between the salivary centers and higher brain centers remain unidentified in humans (Ekström et al., 2011).

Under control of the ANS, salivary glands are innervated predominantly by parasympathetic fibers, but they also receive sympathetic input. Binding of autonomic neurotransmitters with their respective receptors in salivary glands produces a myriad of outcomes and effects. When cholinergic parasympathetic nerves release

acetylcholine (ACh), which binds to muscarinic receptors, saliva is secreted from the acini. Adrenergic (adrenaline and noradrenaline) and cholinergic neurotransmitters are the first messengers of a sympathetic secretory response (Garrett, Ekström, & Anderson, 1999). Sympathetic nerves release noradrenaline to activate adrenergic receptors, which induce smaller volumes of saliva, but with larger amounts of protein, to be expressed from the acini and duct cells (Proctor & Carpenter, 2007). Other neuropeptides released from autonomic nerves can also increase saliva production and alter membrane permeability.

Neural actions on salivary glands include water mobilization, protein secretion, stimulation of cell synthesis, and maintenance of cell function and size. Activation of the parasympathetic and sympathetic systems results in saliva secretions, which interact synergistically to secrete fluid and proteins (Ekström et al., 2011) to meet physiologic demand. Additionally, capillary vessels, which are adjacent to the salivary ducts, indirectly influence saliva secretion. As mentioned earlier, the cranial nerves confer parasympathetic innervation, which causes vasodilation of these vessels. This type of parasympathetic stimulation favors abundant volumes of serous or watery secretions. Conversely, sympathetic innervation is conferred directly by the spinal nerves (i.e., thoracic and cervical), and indirectly by the capillary plexus, which supplies the glands (Garrett, 1987). Indirect input by the capillary plexus is exerted more so by vascular control and not by reflexive sympathetic pathway (Garrett, 1987).

This described model of neuronal control becomes more complicated when considering input from second messenger systems, like cyclic adenosine monophosphate, nitric oxide, and calcium or, neuropeptides such as vasoactive intestinal peptide. Intracellular signaling and co-transmitter receptor activation allow for enhanced coupling between the ANS and the current salivary protein content to create a "real-time" response that ensures the maintenance of optimal saliva composition. Irrespective of the source of stimulation (either parasympathetic or sympathetic), if saliva production is increased, there is a concomitant rise in other salivary ingredients like water, electrolytes, proteins, and other organic molecules (Proctor & Carpenter, 2007).

2.7 Summary

An understanding of the salivary gland anatomy and the regulation of saliva production in a normal state is the first step toward maximizing the power of salivary bioscience. Precise knowledge of gland location can optimize the sampling of analytes. Familiarity with the nuances of saliva production can help to identify sampling confounds (i.e., controlling for flow rates and circadian rhythm) and can also enhance data interpretation. Altogether, this foundational information supports pristine research results and may significantly augment patient-centered care in a clinical setting.

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