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Full Length Article

# Salivary total Immunoglobulin G as a surrogate marker of oral immune activity in salivary bioscience research

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ABSTRACT

The integration of salivary biomeasures in biobehavioral, psychophysiological, and clinical research has greatly expanded our ability to study the biopsychosocial processes underlying health. Much of this research, however, has failed to adequately assess and adjust for the impact of oral immune activity on salivary biomeasure concentrations and associations with serum levels. Aiming to improve the validity and reliability of salivary biomeasure data, we examine salivary total Immunoglobulin G (IgG) as a potential surrogate marker of oral inflammation and immune activity. During a single study visit in Baltimore, Maryland, healthy young adult participants provided matched blood and saliva samples ($N$=99; age 18–37 years, 42% female) and completed an oral health questionnaire. Biospecimens were assayed for total IgG and immune markers related to inflammation (cytokines), blood in saliva (transferrin), and tissue remodeling (matrix metalloproteinase-8). Total IgG (μg/mL) concentrations were higher in serum than saliva. Salivary total IgG was associated with some self-reported oral health measures, and strongly positively associated with all salivary immune markers. Controlling for salivary total IgG may be a feasible, affordable approach to adjusting salivary biomeasure findings for the influence of the oral immune environment when it is not possible or practical to obtain clinical oral health data.

## 1. Introduction

The integration of salivary biomeasures in interdisciplinary research has greatly expanded our ability to examine neuroendocrine, immune, gonadal, and metabolic function using ecologically-valid and minimally-invasive measures. The vast majority of studies using salivary analytes as indices of systemic function, however, have overlooked the potential confounding effects of oral immune activity on salivary analyte variability and associations with physical and mental health conditions. With rates of periodontitis over 40% in the US (Eke et al., 2018), oral inflammation is likely a significant, yet unmeasured, confound in salivary biomarker research.

At a biologic level, oral immune activity may directly alter analyte concentrations in saliva. Early signs of poor oral health include oral inflammation and increased leakage of serum and blood constituents into oral fluids (World Health Organization, 2013; U.S. Department of Health and Human Services, 2000). Along with inflammation-related changes in serum analyte levels in saliva, oral immune activity may directly affect immune and immunosensitive analyte concentrations (e.g., C-reactive protein, cytokines). Furthermore, bacterial load and profiles are likely important factors in both oral disease (Taba et al., 2005) and salivary biomeasure concentrations (e.g., testosterone (Whembolua et al., 2006)).

In addition to biologic effects, there are sociodemographic and environmental factors related to oral immunity that may confound associations between salivary biomeasures and systemic and mental health. As with other health conditions, there are racial/ethnic and socioeconomic disparities in the prevalence of poor oral health in the US (Eke et al., 2018; U.S. Department of Health and Human Services, 2000). There is also substantial covariation between oral and systemic health conditions, health behaviors, and rates of access to and utilization of care

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(Mustapha et al., 2007; Lutfiyya et al., 2019). Thus, the potential confounding effects of oral immune activity in salivary bioscience health research extend beyond simple biologic factors to include a complex set of sociodemographic and environmental factors related to both systemic and oral health.

Controlling for oral health and immunity presents several challenges for salivary bioscientists. Clinical oral health assessments are often not feasible. Ideally, investigators could use either a self-report assessment or low-cost biomarker to control for oral immunity. Anecdotal accounts from dental clinicians suggest that self-reported blood in saliva after oral hygiene practices (e.g., brushing, flossing) is a key symptom of poor oral health. Our work hints that salivary transferrin indexes blood in oral fluid (Kivlighan et al., 2004), and the oral biology literature suggests proinflammatory cytokines and neutrophil collagenase (matrix metalloproteinase-8; MMP-8) may be indices of oral inflammation and connective tissue destruction (Taba et al., 2005; Sorsa et al., 2004; Salminen et al., 2014; Costa et al., 2010; Ghallab, 2018; Nibali et al., 2012; Gursoy et al., 2009; Noack et al., 2017; Sugiyama et al., 2002; Gornowicz et al., 2012). Although more precise than self-report, including multiple oral immune biomeasures as covariates in salivary biomarker studies is likely impractical and cost prohibitive.

We explore the utility of salivary total Immunoglobulin G (IgG) as a standalone surrogate marker of oral immune activity. IgG is the most common antibody in circulation, and, while there is some local production in the oral compartment, most salivary IgG is serum-derived (Brandtzaeg, 2013). IgG protects against infection from pathogens such as viruses, toxins, and bacteria (Doan et al., 2008), and total IgG in saliva may be an indicator of periodontal disease (Taubman and Smith, 1993; Mahanonda et al., 2016; Ebersole, 2003; Kilian et al., 1989). As a primarily serum-derived antibody, salivary total IgG may be uniquely suited to serve as an index of oral inflammation and injury as well as pathogen load. Measuring salivary total IgG is also relatively inexpensive and requires very little sample volume.

Using matched blood and saliva samples from healthy adults, we examine whether variation in salivary total IgG concentrations is unique to the oral compartment and demonstrates associations with other biologic indices of the oral immune environment, including markers of blood (transferrin), tissue destruction (MMP-8), and proinflammatory cytokine activity (interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-alpha (TNFα)). We also examine relations between salivary total IgG and self-reported measures of oral health, hygiene, and access to care.

We expected to confirm higher levels of total IgG in serum than in saliva, and higher levels of salivary total IgG when blood levels in saliva are also high (Schwartz and Granger, 2004). We anticipated total IgG concentrations in saliva are unrelated to serum levels (Challacombe et al., 1995), positively associated with other oral immune markers, and related to self-reported oral health measures.

## 2. Material and methods

The data used in this study were examined previously (e.g., Riis et al., 2017). The study sample, design, and protocols are the same as previously described (see Riis et al., 2017 for details).

### 2.1. Participants

The sample ($N$ = 99; 42% female) was drawn from a single-visit research study of healthy young adults (Table 1) conducted in Baltimore, Maryland in 2012–2013. Study protocols excluded participants reporting medication use (excluding oral contraceptives), acute/chronic illness, open wounds/sores in their mouth, and recent oral surgery.

### 2.2. Procedures

During a 45-minute visit, participants completed a demographic survey, reported on their oral health, hygiene, and access to oral health care, and provided a blood and saliva sample. Participants provided written informed consent and were compensated $50. The university's Institutional Review Board approved all study procedures.

**Table 1**
Participant characteristics and self-reported oral health and hygiene.

| Characteristic | *N* (%)[a] |
|---|---|
| Female | 42 (42%) |
| Age (years) | 23.76 (4.57; 18–37) |
| Body mass index category | |
| Underweight | 4 (4%) |
| Healthy weight | 60 (60%) |
| Overweight | 25 (25%) |
| Obese | 5 (5%) |
| Frequency of flossing (times/week) | 2.07 (2.29; 0–7) |
| Frequency of brushing (times/day) | 1.98 (0.36; 1–3) |
| Bleeding gums after daily oral hygiene | 27 (27%) |
| Access to oral health care | |
| Poor | 2 (2%) |
| Fair | 4 (4%) |
| Good | 21 (21%) |
| Very good | 23 (23%) |
| Excellent | 45 (45%) |
| Oral health problems | 9 (9%) |

[a] For continuous variables (age, flossing and brushing frequency), the mean(standard deviation; and range) are shown.

### 2.3. Blood and saliva collection

Blood was drawn by venipuncture, and additional blood was drawn for serum isolation. Serum was mixed well by inversion and allowed to clot at room temperature (RT) for 30 min (no longer than 2 hours). Unstimulated whole saliva was collected via passive drool. Serum and saliva were aliquoted and stored at −80° C.

### 2.4. Determination of biomeasures

*Total IgG*. Briefly, 0.1 mL of sample was added to each well of a 96-well plate pre-coated with goat anti-human IgG. Plates were incubated for 2 h at RT while mixed continuously at 500 rpm. Following incubation, plates were washed with 1X Wash Buffer and a HRP-linked secondary antibody was added to each well and incubated for 1 hour at RT while mixed continuously at 500 rpm. Plates were then washed, treated with tetramethylbenzidine, and incubated in the dark for 30 min without shaking. A standard plate reader determined optical density at 450 nm. Saliva was plated at a 1:1250 and serum was plated at a 1:500,000 dilution. Linearity on dilution and linearity on spike recovery for IgG was consistent with criterion outlined by Chard (1990). The calibration curve range was 0.78–50 ng/mL. Samples were assayed in duplicate, and saliva and serum samples were plated separately. The average inter- and intra-assay coefficients of variation (CVs) were <6% and <15%, respectively.

*Salivary Total Protein.* Given reported associations between salivary IgG and flow rate (Brandtzaeg, 2013; Nieuw Amerongen et al., 2007), salivary total protein was measured as an indirect indicator of salivary flow rate. Total protein was determined using a commercial assay kit (Pierce™ BCA Catalog #23225, ThermoScientific) following manufacturer procedures. The test range of sensitivity was 20–2000 μg/mL.

*Salivary Transferrin.* Blood leakage into saliva, indexed by salivary transferrin (Schwartz and Granger, 2004), was assayed using a commercially-available kit (Salimetrics, State College, PA; Catalog #1–1302) (Kivlighan et al., 2004). The test range of sensitivity was 0.08–6.6 mg/dL, and the inter- and intra-assay CVs were 4.7% and 2.9%, respectively.

*Matrix Metalloproteinase-8.* Tissue destruction and remodeling in the oral compartment, indexed by salivary MMP-8 (Sorsa et al., 2004), was measured using a commercially-available kit (DuoSet ELISA, R&D Systems, Catalog #DY908) following manufacturer guidelines. The test

range of sensitivity was 62.5–4000 pg/mL, and the inter- and intra-assay CVs were 5.2% and 3.7%, respectively.

Serum MMP-8 was measured using a development kit (DuoSet ELISA, R&D Systems, Catalog #DY908) following manufacturer guidelines and at a 1:25 dilution.

*Cytokines.* Oral inflammation was measured using salivary concentrations of four proinflammatory cytokines associated with oral health (IL-1β, IL-6, IL-8, and TNFα) (Salminen et al., 2014; Costa et al., 2010; Ghallab, 2018; Nibali et al., 2012; Gursoy et al., 2009; Sugiyama et al., 2002; Gornowicz et al., 2012). Serum concentrations of these cytokines were also examined to assess systemic inflammation. Cytokines were measured as part of 9-plex electrochemiluminescence immunoassays following manufacturer protocols (Meso Scale Discovery, Gaithersburg, MD; #R51BB) (Riis et al., 2014). Cytokine lower limits of detection were ≤0.20 pg/mL. Intra-assay CVs were ≤5.0% and inter-assay CVs were <7.5%.

We constructed an oral inflammation composite score using a principal components analysis of the four salivary cytokines. Results supported one underlying component explaining 76% of the variance in salivary cytokine concentrations (standardized component loadings = .83-.92).

### 2.5. Self-reported oral health measures

Participants reported their frequency of flossing and brushing their teeth; if their saliva had a red/pinkish color when they brushed their teeth (yes/no); their level of access to oral health care (Likert-type scale; excellent (5)-poor(1)); and if they had oral health problems (e.g., open cuts/sores, untreated cavities; yes/no). Brushing frequency was dichotomized at twice a day (American Dental Association, 2013). A composite score representing self-reported oral health was constructed by summing responses to these five questions (each rescored/rescaled to a 0–1 scale). This composite score ranged from 1.25 to 5 (M(SD) = 3.62(0.83)) with higher scores representing better self-reported oral health.

### 2.6. Analytic steps

Nine salivary MMP-8 determinations tested higher than the assay upper limit and were replaced with the limit. Six salivary and two serum MMP-8 determinations were excluded due to high intra-assay CVs. Five serum total IgG determinations were missing due to sample quality or type. Most analyte data were positively skewed and kurtotic. Descriptive analyses examined raw analyte data, and we used log-transformed data in statistical analyses that assumed normality. There was ≤5% missing data for self-reported oral health measures.

We compared levels of total IgG in serum and saliva using a paired samples *t*-test and examined the IgG serum-saliva association using a partial correlation controlling for total protein. To assess the impact of blood leakage into the oral compartment on these relations, we compared levels of salivary total IgG in participants with typical ($n$ = 58) vs. elevated salivary transferrin ($n$ = 41; transferrin threshold = 0.5 mg/dL (Schwartz and Granger, 2004)) using an independent samples *t*-test, and we assessed the serum-saliva correlation for total IgG (adjusted for total protein) within these subgroups.

The nature, strength, and specificity of relations between total IgG in serum and saliva and other oral and systemic immune markers were assessed using partial correlations (controlling for total protein). We examined the linearity of these relations using linear regression models with quadratic slope terms and likelihood ratio tests to compare linear and quadratic models.

Separate multivariable linear regression models assessed whether variation in salivary and serum total IgG, and the other oral immune markers, is related to self-reported oral health measures. Given that few participants reported brushing less than twice per day ($n$ = 7), oral health problems ($n$=9), and poor/fair access to care ($n$ = 6), differences in biomeasure concentrations related to these self-reported indices were not examined. Analyses were conducted using Stata SE-15.

## 3. Results

### 3.1. Preliminary analyses and descriptives

Log-transforming analyte concentration data improved the normality of data distributions; transformed analyte data skew ranged from −0.63 to 2.66 and kurtosis ranged from 2.68 to 12.29 with only IL-8 and TNFα in serum exhibiting a kurtosis>6 after transformation. Preliminary analyses found all salivary analytes were positively associated with total protein concentration in saliva ($r$s(92–98)=.35-0.59, $p$s < .01). Therefore, all subsequent analyses examining salivary analytes controlled for total protein. Preliminary analyses also supported our conceptualization of salivary MMP-8, IL-1β, IL-6, IL-8, and TNFα as oral immune indices, as no significant serum-saliva correlations were found for these analytes.

Participant characteristics and a summary of responses to self-report oral health questions are shown in Table 1. Descriptive statistics for analyte data are shown in Table 2.

#### 3.1.1. Total IgG in serum and saliva

Total IgG concentrations were higher in serum than saliva (Table 2; $t(93) = 69.72, p < .001$), and serum total IgG levels were not significantly correlated with salivary levels (partial correlation controlling for salivary total protein: $r = 0.09$, $p = .39$). Salivary total IgG concentrations were higher among participants with considerable blood in their saliva (salivary transferrin≥0.5 mg/dL: M(SD) = 27.56 μg/mL (19.25); transferrin<0.5 mg/dL: (M(SD) = 7.28 μg/ml (4.26), $t(97) = -9.12, p < .001$). However, serum and salivary total IgG were not significantly correlated among participants above or below the 0.5 mg/dL transferrin threshold.

### 3.2. Total IgG and immune markers

Salivary total IgG was positively associated with all other oral immune markers (Table 3). All these relations, except those between salivary total IgG and salivary MMP-8 and the oral inflammation composite score, had significant non-linear slope estimates with steeper positive slopes at lower concentrations and a plateauing of slopes at higher concentrations (linear and quadratic slope estimates for salivary total IgG and: transferrin-$t(98) = 8.89$, $p < .001$, $t(98) = -5.12$, $p < .001$; IL-1β-$t(98) = 6.05, p < .001, t(98) = -3.56, p < .01$; IL-6- $t(98) = 6.57, p < .001$, $t(98) = -5.31, p < .001$; IL8-$t(98) = 5.28, p < .001, t(98) = -2.94, p < .01$; TNFα-$t(98) = 3.82$, $p < .001$, $t(98) = -2.80$, $p < .01$). However, post-estimation model checks and sensitivity analyses that excluded cases with particularly high salivary analyte concentrations (>4 SD from the mean) revealed that non-linear model estimation of salivary total IgG's associations with each of these analytes was especially sensitive to and influenced by cases with high analyte concentrations making non-linear slope estimates tenuous.

There were no significant associations between serum total IgG and the oral immune markers (Table 3), nor between salivary total IgG and serum MMP-8 nor serum proinflammatory cytokines (partial correlations controlling for salivary total protein: $r$s = −0.17-0.01, $p$s > 0.05).

### 3.3. Total IgG and self-reported oral health

Participants who reported red/pink saliva when brushing their teeth had higher concentrations of salivary total IgG (M(SD) = 22.96 μg/ml (19.48)) than those reporting no discoloration (M(SD) = 13.05 μg/mL (14.18); $t(94) = 2.67, p < .01$). Salivary total IgG was also associated with the self-reported oral health composite score with higher salivary total IgG associated with worse self-reported oral health ($t(93) = -2.50$, $p <$ .05). Salivary total IgG was not significantly related to flossing frequency.

Similarly, salivary transferrin and MMP-8 were higher among participants reporting red/pink saliva when brushing their teeth ($t(94) =$ 3.30, $p < .01$; $t(89) = 2.12$, $p < .05$, respectively), and salivary

**Table 2**
Descriptive statistics for biomeasures[a].

| | Mean | Median | SD | Minimum | Maximum | *N* |
|---|---|---|---|---|---|---|
| **Saliva** | | | | | | |
| Total IgG (μg/mL) | 15.67 | 9.92 | 16.22 | 0.40 | 92.60 | 99.00 |
| Total protein (mg/mL) | 700.76 | 624.14 | 364.08 | 148.53 | 1,911.19 | 99.00 |
| Transferrin (mg/dL) | 0.72 | 0.38 | 0.85 | 0.04 | 4.69 | 99.00 |
| MMP-8 (pg/mL) | 76,091.12 | 57,082.00 | 56,001.50 | 5,380.34 | 20,0000.00 | 93.00 |
| IL-1β (pg/mL) | 264.04 | 212.60 | 255.85 | 9.10 | 1,591.19 | 99.00 |
| IL-6 (pg/mL) | 10.09 | 4.07 | 16.72 | 0.35 | 120.93 | 99.00 |
| IL-8 (pg/mL) | 935.77 | 681.84 | 714.76 | 109.11 | 3,374.14 | 99.00 |
| TNFα (pg/mL) | 5.13 | 2.54 | 11.95 | 0.16 | 110.67 | 99.00 |
| **Serum** | | | | | | |
| Total IgG (μg/mL) | 10,158.69 | 9,722.63 | 2,987.00 | 5,510.00 | 23,564.75 | 94.00 |
| MMP-8 (pg/mL) | 29,069.52 | 18,859.52 | 33,156.29 | 2,779.13 | 17,0277.40 | 97.00 |
| IL-1β (pg/mL) | 1.20 | 1.00 | 0.77 | 0.28 | 6.23 | 99.00 |
| IL-6 (pg/mL) | 1.25 | 0.58 | 2.45 | 0.17 | 18.90 | 99.00 |
| IL-8 (pg/mL) | 15.43 | 7.88 | 33.29 | 2.26 | 246.20 | 99.00 |
| TNFα (pg/mL) | 4.77 | 2.46 | 12.55 | 0.93 | 111.72 | 99.00 |

[a] Raw data are presented. SD=Standard deviation; MMP-8 = matrix metallopeptidase-8; IL = interleukin; TNFα = tumor necrosis factor-alpha.

**Table 3**
Associations between total IgG in saliva and serum and salivary oral immune markers.

| Salivary Analytes | Salivary total IgG | Serum total IgG |
|---|---|---|
| | Pearson's *r* | Pearson's *r* |
| Transferrin | .77**§ | .06 |
| MMP-8 | .64** | .05 |
| IL-1β | .57**§ | .02 |
| IL-6 | .66**§ | -.08 |
| IL-8 | .70**§ | -.05 |
| TNFα | .55**§ | -.14 |
| Oral inflammation composite | .73** | -.07 |

*$p < .05$; **$p < .001$ (Bonferroni-corrected α=.007). All analyte data, except salivary MMP-8 are log-transformed. § indicates significant non-linear relations. Associations are adjusted for flow rate using salivary total protein. MMP-8 = matrix metallopeptidase-8; IL = interleukin; TNFα = tumor necrosis factor-alpha. *Ns* = 99 for saliva-saliva and 94 for saliva-serum correlations, except for MMP-8 ($N_{saliva\text{-}saliva}$ = 93, $N_{saliva\text{-}serum}$ = 88).

transferrin, MMP-8, and TNFα were inversely associated with the self-reported oral health composite score ($t(93) = -2.31$, $p < .05$; $t(88) = -2.01$, $p < .05$; $t(93) = -2.25$, $p < .05$, respectively). However, the association between salivary TNFα and the oral health composite score was no longer significant when one participant with particularly high salivary TNFα (110.67 pg/mL) was excluded. All other relations between the oral immune markers and self-reported oral health measures were not statistically significant. There were no significant associations between serum total IgG concentrations and the self-reported oral health measures.

## 4. Discussion

Our findings support the notion that salivary total IgG may serve as a surrogate index of the oral immune environment when it is not possible to obtain clinical assessments of oral health and inflammation. Salivary total IgG was positively associated with all the oral immune markers examined and increased among participants reporting worse oral health. Salivary total IgG variability was also specific to the oral compartment and not related to systemic total IgG nor markers of systemic inflammation. Importantly, serum and salivary total IgG levels were not significantly correlated even among participants with higher levels of blood in their saliva. While other studies have shown the independence of serum and salivary IgG in both heathy and diseased samples (Challacombe et al., 1995; Patidar et al., 2011; Kandasamy et al., 2016), this finding is especially salient given that IgG in saliva is primarily serum-derived, entering the oral compartment via the gingival crevice (Brandtzaeg, 2007, 2013). It is important to note, however, that gingival tissues contain IgG-secreting B lymphocytes and plasma cells (Mahanonda et al., 2016; Ebersole, 2003; Kilian et al., 1989), and it is estimated that up to 20% of IgG concentrations in crevicular fluid may represent local IgG production (Challacombe et al., 2015). While the origins of total IgG in our samples of whole saliva cannot be completely delineated, our findings suggest coordinated immune function in the oral cavity involving inflammatory, tissue remodeling, and IgG antibody processes. Taken together, our findings raise the possibility that salivary total IgG may serve as an index of the oral immune environment among individuals with varying degrees of oral health.

As noted previously, IgG is a central component of oral mucosa immunity protecting the body from infection and disease. IgG levels increase during both the primary response and the more intense secondary response when B-cells are exposed to antigens (viruses, toxins, bacteria, and fungi) (Doan et al., 2008). Many soluble signals involved in initiating and maintaining the immune response are produced, but the levels of most of these signals decline rapidly after exposure. By contrast, IgG levels remain elevated for an extended period after antigen exposure (Doan et al., 2008). The protracted elevation of IgG post-exposure, combined with our understanding that the immune system in the oral compartment is subject to high rates of antigen exposures (Dewhirst et al., 2010), underscores the potential utility of salivary total IgG as a surrogate biomarker of the general state of oral immunity (in contrast to more specific markers such as cytokines).

We also found significant non-linear relations between salivary total IgG and many other oral immune markers. All non-linear relations were characterized by steeper, positive slopes at lower analyte concentrations and a plateauing of slopes at higher analyte concentrations. These non-linear relations may suggest a potential saturation point at which the physiological relationship between analytes begins to break down. These findings both support the notion that relations between salivary analytes vary by the nature of the oral immune environment, and underscore the need for appropriate estimation of salivary analyte relations. Non-linear associations may be misspecified or overlooked with traditional linear modeling. These results also highlight the need for additional studies examining oral immune processes among healthy, at-risk, and clinical populations. It is unclear if our non-linear findings reflect the true nature of the physiologic processes, or if they are driven by extreme data points. All non-linear relations observed in our study were sensitive to cases with high salivary analyte concentrations, and our sample did not have enough participants with higher analyte concentrations to reliably estimate non-linear slopes. Given our healthy and relatively homogenous sample, additional research is needed to confirm and extend our findings in samples reporting both systemic and oral health problems. Future studies should also explore measurement and modeling approaches for

salivary total IgG that provide the most efficient adjustment for oral immune activity (e.g., are there thresholds that indicate disease and/or confounding?; is statistical covariation sufficient for the meaningful interpretation of adjusted findings?).

Additional research examining a broader range of biologic and behavioral factors associated with oral immunity and health is also needed. Our sample of healthy young adults had too few participants endorsing smoking and recent medication use to examine the effects of these behaviors, and we did not have information about other important biologic factors and health behaviors, such as measures of anti-inflammatory cytokine activity, bacterial/viral load, oxidative stress, and eating behaviors (Taba et al., 2005; Salminen et al., 2014; Ghallab, 2018). The relative importance of these factors as confounders in salivary bioscience studies may vary by sample characteristics (e.g., age, health) and analyte of interest. To maximize their impact, future studies should be analyte-specific and focus on delineating significant oral immune and health confounds for individual salivary biomeasures.

Finally, it is important to recognize that the conceptualization and measurement of oral health has been studied extensively in fields such as oral biology, periodontology, and oral public health using both biologic and self-report tools (World Health Organization, 2013; U.S. Department of Health and Human Services, 2000; Zaura and Ten Cate, 2015; Fisher-Owens et al., 2007; Baker et al., 2008). Drawing upon this work, future researchers should test the feasibility and utility of incorporating comprehensive self-report measures of oral health into their studies. There are many tools available to assess oral health (e.g., World Health Organization, 2013; Fisher-Owens et al., 2007), and these are not typically used in salivary bioscience health-related field and experimental research. The self-reported oral heath questions examined in this study are similar to those used in other salivary bioscience studies. The inconsistent associations between our salivary immune markers and responses to these questions suggest that these measures should be revised or replaced with validated measures. Self-report tools that specifically capture the confounding effects of oral immune activity on salivary analyte variability are also needed.

## 5. Conclusions

Given its strong associations with biologic indices of oral inflammation, tissue degradation and remodeling, and blood leakage into saliva, salivary total IgG may be a viable surrogate marker of oral immune activity. Salivary total IgG may be a particularly valuable oral immune marker for studies with limited resources and for those interested in immune and immunosensitive analytes and/or analytes that enter saliva via circulation. Additional research comparing salivary total IgG, and other biologic and self-reported measures of oral health and immunity, to a “gold standard” (i.e., clinical assessments) is needed to identify a true oral immune biomarker.

## Declaration of competing interest

In the interest of full disclosure, DAG is founder and chief scientific and strategy advisor at Salimetrics LLC and Salivabio LLC, and 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. JLS is a Research and Development Scientist at Salimetrics.

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