The Biology of Social Adversity in Oral Disease

by

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Abstract

Several pathways have been proposed to explain the relationship between adverse social and living conditions and oral health outcomes. However, little is known about the underlying biological mechanisms by which the social “gets under the skin” to bring about oral diseases. This work aims to investigate the pathobiological pathways by which adverse social exposures (focusing on socioeconomic position), become embodied in oral and systemic inflammation. The specific objectives are to: (1) evaluate the evidence on social-biological interactions in oral disease; (2) assess the contribution of socioeconomic factors to the association between periodontal and systemic inflammation; (3) assess socioeconomic differences in periodontal and oral immune parameters; and (4) examine the contribution of psychosocial stress factors and the activation of the stress pathway in these relationships. To do this, we first conducted a systematic review, based on which a conceptual model was plotted, followed by an analysis of secondary NHANES IV data where the contribution of socioeconomic position to the relationship between periodontal and systemic inflammation was assessed. Finally, an analysis of primary data was conducted to assess the socioeconomic differences in periodontal disease and oral immune outcomes and the contribution of financial stress, perceived stress and the stress hormone
cortisol to these differences. Overall, the results showed that socioeconomic factors attenuate the relationship between periodontal disease and systemic inflammation, and that individuals on the lower rungs of the socioeconomic hierarchy are at a greater risk for a pro-inflammatory oral immune system that potentially increases their vulnerability to periodontal tissue damage. This relationship was significantly attenuated by the effect of financial and perceived stress and cortisol, indicating a biopsychosocial pathway between socioeconomic exposures and periodontal disease. Collectively, the results from this work show that the socioeconomic, psychosocial and biological factors have a convergent role in the oral disease process. Integrated policy solutions that target these underlying factors in oral disease and associated inequalities are required.
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To my everything, Adham and Lina. This, and all that follows, I do for you. Believe that with passion and hard work, dreams do come true!
“Disease is not something personal or special, but only a manifestation of life under modified conditions, operating according to the same laws as apply to the living body, from the first moment until death.”

– Rudolf Virchow
“A caricature of some social epidemiology would be that it has spent too much time relating an indicator of social structure such as income, education or occupation to mortality or other health outcomes without asking why. The research task is to give an account of what links social structure to health outcomes — to ask, what are the intermediary steps?”

– Sir Michael Marmot
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropin hormone</td>
</tr>
<tr>
<td>AGP</td>
<td>Aggressive periodontitis</td>
</tr>
<tr>
<td>AL</td>
<td>Attachment loss</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BOP</td>
<td>Bleeding on probing</td>
</tr>
<tr>
<td>CAL</td>
<td>Clinical attachment loss</td>
</tr>
<tr>
<td>CGP</td>
<td>Chronic generalized periodontitis</td>
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<tr>
<td>CHMS</td>
<td>Canadian Health Measures Survey</td>
</tr>
<tr>
<td>CIL</td>
<td>Cumulative inflammatory load</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>dmfs</td>
<td>Decayed, missing and filled surfaces (primary teeth)</td>
</tr>
<tr>
<td>DMFS</td>
<td>Decayed, missing and filled surfaces (permanent teeth)</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DSM-IV</td>
<td>Job-related stress depression questionnaire</td>
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<tr>
<td>EOP</td>
<td>Early onset or aggressive periodontitis</td>
</tr>
<tr>
<td>FSS</td>
<td>Financial stress scale</td>
</tr>
<tr>
<td>G</td>
<td>Gingivitis</td>
</tr>
<tr>
<td>GAS</td>
<td>General Adaptation Syndrome</td>
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GC    Glucocorticoids
GCF   Gingival crevicular fluid
GI    Gingival index
GOHIRA Global Oral Health Inequalities Research Agenda
GOHIRN Global Oral Health Inequalities Research Network
HC    Hair cortisol
HDL   High-density lipoprotein
HPA   Hypothalamic pituitary adrenal
IADR  International Association for Dental Research
IL    Interleukin
IRR   Incidence rate ratio
MAPS  Modified and perceived stress scale
MFI   Mean Fluorescence Intensity
MMP   Matrix metalloproteinase
NHANES IV National Health and Nutrition Examination Survey IV
NLR   Neutrophil to lymphocyte ratio
NPT   Non-surgical periodontal treatment
OD    Oral disease
OIL   Oral inflammatory load
OPMN  Oral polymorphonuclear leukocytes (oral neutrophils)
PD    Periodontal disease
PHAC  Public Health Agency of Canada
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PI</td>
<td>Plaque index</td>
</tr>
<tr>
<td>PIR</td>
<td>Poverty to income ratio</td>
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<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocytes (peripheral neutrophils)</td>
</tr>
<tr>
<td>PRD</td>
<td>Probing depths</td>
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<tr>
<td>PROINF</td>
<td>Proinflammatory immunophenotype</td>
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<tr>
<td>PSS</td>
<td>Perceived stress scale</td>
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<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>R</td>
<td>Reinforcing loops</td>
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<tr>
<td>r</td>
<td>Spearman’s rank correlation coefficient</td>
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<tr>
<td>sAA</td>
<td>Salivary alpha-amylase</td>
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<tr>
<td>SAM</td>
<td>Sympathetic-adrenal-medullary</td>
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<tr>
<td>SEP</td>
<td>Socioeconomic position</td>
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<tr>
<td>SHIP</td>
<td>Study of Health in Pomerania</td>
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<tr>
<td>sIgA</td>
<td>Salivary immunoglobulin-A</td>
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<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
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<tr>
<td>SUP</td>
<td>Suppuration</td>
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<tr>
<td>Th</td>
<td>T-helper cells</td>
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<td>US</td>
<td>United States</td>
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<tr>
<td>WBC</td>
<td>White blood cells</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1

Introduction and Dissertation Overview

Despite being largely preventable, oral disease remains a major global public health problem that continues to place a significant negative impact on individuals and society (1). This burden is known to disproportionately concentrate in socioeconomically marginalized groups and worsens as one travels down the social hierarchy (2). In Canada, for example, untreated dental caries and periodontal disease (PD) are worse among those with lower income and education, those without dental insurance, as well as among immigrants and those who self-identify as Aboriginal (3). Recognizing these oral health inequalities has led oral health research and policy entities in Canada and around the world to emphasize the importance of developing novel “upstream” strategies that can mitigate oral disease and target its burden in vulnerable populations (2, 4, 5). Surely, devising such strategies not only requires an understanding of the social patterning in oral disease, but importantly the pathways and the intermediary steps involved (2).

Several theoretical pathways and conceptualizations have been postulated as to the link between the structural and social environment and oral health outcomes. While a substantial body of evidence exists on the social drivers of oral disease, little is actually known on the biological pathways by which these social factors can “get under the skin” to bring about oral disease and related oral health inequalities (6).

The impetus for this dissertation was the need to develop an understanding of the biological mechanisms by which social and living conditions can become embodied,
afflicting oral disease and associated inequalities. Lying along the nexus of the social and biomedical sciences, this work employs interdisciplinary methods from social epidemiology, behavioural sciences and immunology to investigate the role of adverse socioeconomic exposures in oral disease and related oral and systemic immune processes. Some of the research questions this work tackles are: What are the potential pathways linking the social to biological factors in oral diseases? To what extent do adverse social exposures contribute to periodontal inflammation and related pathobiological processes? To what extent do psychosocial stress factors and the stress response contribute to the social differences in periodontal inflammation?

Researchers concerned with the biology of social adversity have suggested that due to the complexity of the biological embodiment process, conclusions need be drawn from a range of study designs that are conducted in different populations, thus bolstering and supporting each other’s findings (7). As such, this dissertation comprises three studies that aim to address the questions above. The first study reviews the role of social factors in the pathobiology of oral disease, focusing on socioeconomic exposures, to develop a better understanding of the pathways therein and to identify knowledge gaps in this area of study. It then introduces a conceptual model of the social-biological interactions in oral and systemic health, which demonstrates how social and biological factors are reciprocal and dynamic in nature, highlighting the under-investigated role of the biopsychosocial pathway in oral health.

The second is a cross-sectional study that utilizes a population-based sample from the American National Health and Nutrition Examination Survey (NHANES IV) to examine the role of socioeconomic factors such as the poverty-to-income ratio and
educational attainment in the association between PD and systemic inflammation. The results show that socioeconomic disadvantage can significantly contribute to these relationships.

Finally, the third study utilizes primary data to examine the biopsychosocial pathway in PD and immune processes. It investigates the differences between socioeconomic groups in PD and oral immune parameters, and whether these are explained by psychosocial stress factors and the activation of the body’s stress pathway. Its findings show that higher levels of psychosocial stress indicators, such as financial stress, perceived stress, and an activation of stress pathways via the stress hormone cortisol are associated with increased PD levels and a greater risk for a pro-inflammatory oral immunity, significantly explaining the socioeconomic differences observed in these outcomes.

Collectively, the work from this dissertation emphasizes that adverse socioeconomic exposures are linked to oral disease and oral inflammatory processes. It demonstrates that the psychosocial pathway is an important player in these relationships, thus implicating the need for integrated policy solutions that target the underlying social determinants of oral disease and associated inequalities in oral health.
Chapter 2

Literature Review

2.1 Oral diseases and oral health inequalities

“Thee – thou hell o’ a’ [of all] diseases,” was how the poet Robert Burns depicted his agonizing pain in his poem “Address to the Toothache” (8, 9) (Figure 2-1). Over two hundred years later, and despite the unprecedented advances in its prevention, diagnosis and treatment, oral disease continues to place a toll on individuals and societies (10). The main oral diseases, dental caries and PD, are some of the world’s most common chronic conditions, affecting nearly half of the world’s population (1, 10-14). They also pose a significant economic burden due to the associated costs of treating them and/or leaving them untreated (15). For example, being a major cause of tooth loss, PD constitutes around 37 per cent of worldwide losses in productivity (15). Additionally, its strong association with various chronic inflammatory health conditions (e.g. cardiovascular disease (CVD) and diabetes) has been suggested to add to its imposed morbidity.

The burden of oral disease disproportionately affects socially disadvantaged individuals and groups, creating significant oral health inequalities (16). For example, the Canadian Health Measures Survey has shown that dental caries, periodontal conditions and oral pain concentrate in lower income individuals, who typically lack dental insurance and decline dental care due to cost (17). Being a universal phenomenon that exists along the life-course, oral health inequalities are not simply differences between the socially ‘advantaged’ and ‘disadvantaged’, but have been shown to follow a stepwise gradient, with oral health being worse as one goes down each of the rungs on the social hierarchy (Figure
These socioeconomic differences are similar to those observed in general health outcomes (Figure 2-3) (18). Furthermore, inequalities are observed among adults in Canada, with more deprived groups having more decayed and missing teeth and oral pain and fewer filled teeth than the more affluent (20).

The Ottawa Charter for Health Promotion states that health is not the product of medical or other conditions directly related to health, but an outcome determined by a complex of social and environmental factors (21). This view of health was also emphasized by the World Health Organization Commission on the Social Determinants of Health in its call for developing interventions that target the ‘upstream’ structural and social causes of disease and health inequalities (5). Several oral health research and policy bodies have combined this with a call for shifting towards interdisciplinary research that would integrate the social and biological sciences to help understand the causes of disease (2, 22). This was demonstrated in the International Association of Dental Research-Global Oral Health Inequalities Research Network (IADR-GOHIRN) agenda, released in 2013, which prioritized the need for developing an understanding of the full range of the biological, behavioural, psychosocial and social determinants of oral health and well-being (23). Indeed, an integrative view of how this array of factors interplays is important for evidence-informed public health policies that aim to mitigate oral disease and oral health inequalities (10, 23).
Figure 2-1. Illustration of “Address to the toothache” by William Brassey Hole (24).
Figure 2-2. Social inequalities in oral health. Source: Unknown
Figure 2-3. Socioeconomic gradients in perceived oral and general health outcomes, periodontitis and ischaemic heart disease Source: Sabbah, et. al (2007) (18).
2.2 A false dichotomy: Is oral disease social or biological?

Several conceptualizations have been postulated to understand the causes of oral disease and the pathways to its inequitable distribution. These have ranged from ‘proximal’ biomedical and behavioural models to ones that included the more ‘distal’ determinants of health, such as the fundamental causes model. Much of the current research on oral disease and related inequalities tends to focus on either sides of the equation (i.e. strictly biomedical or strictly social) (25). Such segregation has created a false dichotomy between the social and biological factors that are likely to interplay in health and disease (26). The next section describes the concepts behind some of the models used to explain oral disease and oral health inequalities, alongside some of their strengths and shortcomings. Importantly, it highlights the need for an integrative approach to understanding oral disease and associated inequalities.

2.2.1 Biomedical and behavioural models

Biomedical and behavioural models have traditionally predominated the oral disease prevention and treatment paradigms. The etiology of PD, for example, is often referred to as the result of an interaction between host immune-inflammatory responses, the pathogenic oral biofilm and environmental and acquired risk factors (e.g. smoking, poor oral hygiene) (Figure 2-4) (27). Likewise, dental caries is often narrowly seen as the biological interaction between microbial, dietary and tooth-related factors (e.g. enamel thickness and composition) (28).
Taking a ‘reductionist’ approach, explanations that focus on biomedical and behavioural models have been deemed to only tackle the individual micro-level, resulting in downstream, arguably ineffective interventions (e.g. oral health education) that yield unsustainable results, and possibly even widen the social gaps in oral health. (29, 30) This has been attributed to a number of reasons, including its failure to address the underlying causes of oral disease, acknowledging behaviours as causes rather than mediators. (31) Also, this approach has been described as “victim blaming” in nature in that it tends to blame the less affluent for their poor oral health status by implying their “need to take better care of their teeth” or generally attributing unfavorable health conditions to the “plight of the poor” (32, 33). Additionally, it tends to isolate the mouth from the rest of the body, thus compartmentalizing oral disease rather than integrating it with other health outcomes (34).

2.2.2 The fundamental causes model

The fundamental causes model by Link and Phelan, is one of the most cited models that has had a significant impact on social epidemiological conceptualizations of the causes of health and disease (35). It suggests that health inequalities occur due the unequal distribution of knowledge, power, prestige and resources among groups in society, placing socially disadvantaged individuals at the “risk of risk” (36, 37). These underlying causes apply to oral health inequalities, where oral disease shares common risk factors with other chronic health conditions (34, 38). While the fundamental causes theory has driven an understanding of the social risk factors of ill-health, it has been suggested to lack the specifics on the links or the mechanisms between adverse social exposures and health
outcomes. In other words, it does not elaborate on the complexity of the dynamic relationships between social and biological factors (25, 26, 39). It has thus been suggested by some to have unintentionally promoted the false dichotomy between the social and biological, and may have indirectly given biomedical researchers and clinicians the notion that it may be possible to ignore the social factors and intervene only at the level of the ‘proximal’ causes of disease (26, 39). Thus, despite repeated calls to incorporate social factors into health research, the fundamental causes model might have not changed how biomedical research is conducted and how biological questions are framed and answered (26).
Figure 2-4. Schematic illustration of the pathogenesis of periodontitis. Based on Carranza’s Clinical Periodontology, 10th Ed. WB. Saunders Company; 2011 (40).
2.2.3 The biopsychosocial model

The biopsychosocial model was introduced by Engel in 1977, offering an alternative to the prevailing reductionist biomedical model (41). Engel critiqued the narrow focus taken by biomedical and behavioural approaches. Some of these critiques included:

- A biochemical alteration does not translate directly into an illness; the appearance of illness results from the interaction of diverse causal factors, including those at the molecular, individual, and social levels;
- Psychosocial variables are more important determinants of susceptibility, severity, and course of illness than what a biomedical view of illness maintains;
- The success of most biological treatments is influenced by psychosocial factors (42).

As such, the biopsychosocial model proposed that, in addition to the biological factors in the biomedical model of illness, psychological (entailing thoughts, emotions, and behaviours) and social (socioeconomic, environmental and cultural) factors also play a significant role in the context of disease (Figure 2-5) (42, 43). Incorporating the social and psychosocial dimensions in understanding disease has shown that they play an important role either directly through the effect of stress on pathophysiology and/or indirectly by influencing health behaviours (42). This delineation of the pathways linking the social to the biological is significantly relevant to health policy as it establishes causality and ultimately provides evidence for consumers of health research to guide interventions (44).
2.2.3.1 Biological embodiment: how the social “gets under the skin”

The biopsychosocial pathway is the route by which adverse social and economic exposures can become embodied or “get under the skin” to afflict disease (45). Krieger defines the notion of embodiment as “how we, like any living organism, literally incorporate, biologically, the world in which we live, including our societal and ecological circumstances” (46). In recent years, biological embodiment has become an organizing framework for much of the research on social-biological interactions (46).

Despite its complexity, evidence on biological embodiment has been demonstrated in several studies, where the influence of social adversity and other social exposures has been documented across a number of health outcomes. These include cardiovascular, metabolic, immune and neuroendocrine functions (35, 47). The Whitehall Study II investigated the psychosocial causes of CVD inequalities in British civil servants (48). Here, individuals in lower occupational ranks showed higher stress-related neuroendocrine activity as demonstrated by the increased urinary output of metabolites of the stress hormones cortisol and catecholamines (49). Low heart rate variability, an indicator of adverse autonomic (sympathetic-parasympathetic) balance, was also shown to associate with low employment grade (50). Recent work in this area conducted in children has shown that childhood exposure to social disadvantage can lead to an altered brain structure and function, exacerbated proinflammatory processes and changes in behavioural tendencies. Interestingly, similar findings were observed in animal studies on social stress. In his work on the behaviour and physiology of wild baboons, Robert Sapolsky showed that the males in the troop were either dominants or subordinates; the latter having higher
levels of the stress hormone cortisol, which inversely correlated with high-density lipoprotein (HDL), a protecting factor against CVD (51).

**Figure 2-5.** The biopsychosocial model in health incorporates social and psychological factors along with the biological ones.
In terms of oral health, a significant body of research has examined the effect of various stressors on oral health, oral health behaviours and oral health-related quality of life (52-55). Work conditions, for instance, such as control over work time, routinization and work flexibility have been shown to associate with oral health-compromising behaviours such as the frequency and effectiveness of tooth cleaning (56). Low levels of marital quality and the experience of negative life events have been shown to relate to PD as well (57). In one study using a representative sample of the Australian adult population, gender was shown to modify the relationship between perceived stress and oro-facial pain (55). However, fewer studies have examined psychosocial stress as being directly related to the social determinants of oral health. In a study by Genco et al., financial strain was associated with loss of attachment and alveolar bone loss, which was exacerbated by inadequate coping strategies (58). The effect of the psychosocial environment on oral health was shown to apply to children as well, where parental financial stress, children’s cortisol and oral bacterial counts were shown to interact in predicting carious lesions in children (59).

To this end, it is clear that the role of psychosocial stress in the relationship between adverse social exposures and oral health is recognized, and while it has recently become an area of interest and research, it remains understudied. Some of the conceptualizations by which psychosocial stress can become embodied in oral and general health outcomes are outlined in the next section.
2.2.3.2 Conceptualizations of biological embodiment

Building on the observed associations between psychosocial stress and biological outcomes in health inequalities, two concepts have been proposed that emphasize the impact of stress on multiple biological systems; *allostatic load* and *weathering*.

Allostatic load is the cumulative ‘wear and tear’ on different body systems due to the exposure to chronic stress (60). When psychological demands exceed an individual’s capacity to adapt physiologically and emotionally, this cumulative dysregulation compounds over time, constituting a disease risk and eventually triggering chronic disease (61). Indeed, allostatic load has been shown to increase the risk for a number of health conditions, including CVD, cognitive and physical decline and all-cause mortality (62-65).

A number of parameters have been used to measure allostatic load. McEwen proposed a summative score where markers such as systolic and diastolic blood pressure, waist-to-hip ratio, serum HDL and total cholesterol, glycated haemoglobin, and overnight urinary cortisol secretion can be used as an aggregate score (60, 66). These measures have been used with the aim of attaining a range of values for markers that include the different regulatory systems involved in adapting to allostatic load (66, 67). Allostatic load has been suggested to have an additional predictive power in disease risk over individual biomarkers and provides a potential understanding of the physiological burden imposed by exposure to detrimental stressors. Recently, allostatic load has also been linked to brain structure and function, where chronic stress affecting the prefrontal cortex, amygdala and hippocampus results in the dysregulation the hypothalamic pituitary adrenal (HPA) and sympato-adrenal medullary (SAM) axes and correlates with alterations in cardiovascular, metabolic
and inflammatory parameters (67, 68). The cumulative allostatic load model was also studied in relation to oral health in studies using data from the American population. Their results showed that socioeconomic and racial differences in PD are explained by allostatic load as an indicator of stress (69, 70).

The weathering hypothesis postulates that the timing for developmental milestones or biological events occurs earlier due to adverse social exposures (71). Initially proposed to explain racial inequalities in high rates of teen pregnancies (71), the weathering hypothesis was carried forward to explain the occurrence of several inequalities in health. Further studies on weathering suggested that the cumulative biological impact of being chronically exposed to socially-structured stressors accelerates aging in marginalized populations, leading to health vulnerability and possibly shorter life expectancies (72, 73). An example of this is the shortened leukocyte telomere lengths observed in association with lower socioeconomic status, unstable family structure and exposure to harsh parenting (74). With regard to oral health, a few studies have examined telomere-length shortening as a sign of biological aging in individuals with PD (75, 76). However, the weathering concept in relation to social disadvantage has not been studied in oral health conditions.

2.2.3.3 The road to inflammation is paved with stress

Employing a biopsychosocial model to social and biological interactions in disease requires an understanding of the underlying pathobiological mechanisms. Hans Selye, known as the father of the stress field, first defined stress as “the non-specific neuroendocrine response of the body” (77, 78). Realizing that almost every other system was involved in this response, he later dropped the term “neuroendocrine”(79, 80). As his discoveries were the
beginning of an understanding of how psychosocial stress can be pathological, he described
a consistent three-stage pattern that he referred to as the General Adaptation Syndrome
(GAS), later renamed as the ‘stress response’ (79). In the first stage, the alarm reaction, the
body prepares to fight or flee, followed by a stage of resistance, where the various body
systems prepare for sustained attack against the stressor. In this second stage, the immune
response continues to increase and the body adapts to the specific stressor. For example, if
the stressor is nutritional deprivation, the body may become lethargic to conserve energy
while the absorption of nutrients is maximized. Finally, in the third stage, exhaustion, the
systems become worn out and resistance to the stressor cannot be sustained (80). Selye
identified several specific hormones involved in the stress response, in particular,
glucocorticoids (GC) (81). His central point was that the prolonged effect of stress would
have a negative impact on health, ultimately resulting in what was termed as the ‘wear and
tear’ or the allostatic load concept, described above (66).

Later research in psychoneuroimmunology and the pathobiology of stress has
shown that the stress response is characterized by the activation of the HPA and SAM axes
and the sympathetic nervous system (SNS) (82). HPA hormones regulate inflammation,
and the extent of this regulation depends on the nature and the severity of the stimulus, i.e.
chronic or acute (83). The activation of the HPA results in the secretion of the
corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the
hypothalamus into the hypophyseal portal blood supply, stimulating the expression of the
adrenocorticotropin hormone (ACTH) in the anterior pituitary gland (83). ACTH circulates
in the bloodstream to the adrenal glands inducing the expression and the release of GCs,
such as cortisol (84). The latter regulates immunity through binding of the hormone to its
receptor on the immune cell surface and by inducing the dysregulation of cytokine production (84, 85).

The effect of cortisol on immunity has been shown to vary depending on type and length of the stress exposure. Generally, cortisol is an immunosuppressant, which inhibits Th1 cytokines (e.g. interferon-γ, interleukin-2), shifting the cytokine production to the anti-inflammatory Th2 responses (e.g. interleukins-4 and 10) (85). Additionally, it is known to suppress maturation, differentiation, and proliferation of immune cells including innate, T-cell, and B-cell functions and chronic allergic reactions (85). Experimental studies have shown that cortisol regulates inflammatory cell populations, inversely correlating with lymphocytes, but positively with neutrophils, where it has been shown to impair apoptosis and increase neutrophil half-life (86). Meanwhile, cortisol is also known to reduce cell-cell adhesion and impair circulating neutrophil function (e.g. superoxide generation), contributing to infection (87). This explains observations from several studies showing that the overstimulation of the HPA axis is correlated with the increased susceptibility to viral infections, prolonged wound healing and decreased antibody production after vaccination (88-90). However, HPA overstimulation has also been linked to inflammatory disease possibly characterized by a hyper-stimulated immune system. This suggests that there are stress-related changes in the sensitivity of target systems to GC regulation (87). For example, disruption of leukocyte distributional sensitivity to GC and diminished glucocorticoid receptor mediated gene expression have been linked to chronic stress and HPA baseline activity, in both animal and human models (91)(92-95) The pathways by which the stress response may induce oral/periodontal inflammation are illustrated in Figure 2-6.
The above concepts and pathways reveal how the social and psychosocial environment influences the various body systems involved in the stress response. However, whether similar mechanisms take place in oral disease and whether they may be the link by which structural factors become transduced to biological ones in oral health outcomes remains unclear.
Figure 2-6. Illustration of the potential mechanisms of biological embodiment in PD. Stimulation of the HPA and SAM axes results in the production of the stress hormones glucocorticoids (cortisol) and catecholamines (adrenaline and noradrenaline) from the adrenal gland. These can modulate neutrophils in the circulation by binding to their respective receptors. Transmigration into the periodontal tissues then occurs, possibly contributing to periodontal tissue damage (Gomaa et al; unpublished work).
Chapter 3

Rationale, Objectives and Hypotheses

The importance of understanding the biology of social adversity primarily stems from the ability to provide evidence on the impact of the structural and social environment on health outcomes from standpoints that are relevant to various disciplines, including the biomedical and social sciences, thus building bridges between disciplines. The work presented in this dissertation thus advances knowledge on the pathobiological processes responsible for oral disease and their driving social factors. With the biomedical and behavioural “downstream bias” in formulating research questions that attempt to understand oral health and disease, this work contributes to a paradigm shift to the more comprehensive “upstream” understanding of oral disease, which takes the social and psychosocial factors into consideration.

Investigating the processes by which social adversity “gets under the skin” to bring about disease has been postulated as necessary for guiding interventions, identifying risk factors, and motivating policy and political action (47). Such understanding is essential for public health policy makers and other consumers of health research aiming to devise targeted and/or universal interventions that can alleviate the burden of disease in the population and narrow social gaps in oral health.

Due to the multiple factors at play in the biology of social adversity, researchers have recognized its complexity and highlight that identifying risk factors and furthermore causality may not be a straightforward process. As such, it is postulated that a key strategy in advancing this field is the use of different study designs, pointing out that triangulating
across studies can provide stronger evidence on the biological mechanisms linking social exposures to health outcomes.

Stemming from this, the overarching aim of this work is to investigate the concept of biological embodiment in oral disease and to develop a better understanding of how structural and social factors are linked to biological ones, manifesting as oral disease. In doing so, this dissertation comprises three studies. The specific objectives and hypotheses for each of these are outlined below.

3.1 Study I: Social-biological interactions in oral disease: A “cells to society” view

Objectives

1. To evaluate the literature on the relationship between social and psychosocial conditions and the pathobiological processes in OD.
2. To develop a conceptual model of the social-biological interactions in OD.

3.2 Study II. How does the social “get the under the gums”? The role of socioeconomic position in the oral-systemic health link

Objectives

1. To assess the extent of the association between PD and systemic inflammation;
2. To determine whether socioeconomic position (SEP) explains this relationship.

Hypotheses

1. There are socioeconomic differences in PD and systemic inflammation;
2. Biomarkers of systemic inflammation will be associated with increased levels of PD;
3. The PD-systemic inflammation relationship will be explained by socioeconomic factors.

3.3 Study III. Stressed-out immunity: A gateway from social adversity to periodontal disease?

Objectives
1. To assess the socioeconomic differences in PD and oral immune parameters;
2. To assess psychosocial stress and stress hormones in relation to PD and oral immune parameters;
3. To examine the contribution of psychosocial stress and stress hormones to the socioeconomic differences in PD and oral immunity.

Hypotheses
1. Lower socioeconomic position will be associated with higher levels of PD, oral neutrophil counts and proinflammatory oral neutrophils;
2. Financial and perceived stress and higher levels of stress hormones will be associated with higher levels of PD and oral neutrophil counts and proinflammatory oral neutrophils;
3. Financial and perceived stress and stress hormones will mediate the socioeconomic differences in PD and oral immunity.
Chapter 4

Study I: Social-Biological Interactions in Oral Disease: A ‘Cells-to-Society’ View

This study was published in PLOS ONE:


Abstract

Oral diseases constitute a major worldwide public health problem, with their burden concentrating in socially disadvantaged and less affluent groups of the population, resulting in significant oral health inequalities. Biomedical and behavioural approaches have proven relatively ineffective in reducing these inequalities, and have potentially increased the health gap between social groups. Some suggest this stems from a lack of understanding of how the social and psychosocial contexts in which behavioural and biological changes occur influence oral disease. To unravel the pathways through which social factors affect oral health outcomes, a better understanding is thus needed of how the social ‘gets under the skin,’ or becomes embodied, to alter the biological. In this paper, we present the current knowledge on the interplay between social and biological factors in oral disease. We first provide an overview of the process of embodiment in chronic disease and then evaluate the evidence on embodiment in oral disease by reviewing published studies in this area. Results show that, in PD, income, education and perceived stress are correlated with elevated levels of stress hormones, disrupted immune biomarkers and increased allostatic
load. Similarly, socioeconomic position and increased financial stress are related to increased stress hormones and cariogenic bacterial counts in dental caries. Based on these results, we propose a dynamic model depicting social-biological interactions that illustrates potential interdependencies between social and biological factors that lead to poor oral health. This work and the proposed model may aid in developing a better understanding of the causes of oral health inequalities and implicate the importance of addressing the social determinants of oral health in innovating public health interventions.
4.1 Introduction

Oral diseases are some of the most common chronic conditions around the world (14). Despite the advent of preventive and therapeutic dentistry, oral diseases continue to place a heavy toll on socially disadvantaged groups, creating persistent social gaps in oral health (96). In Canada, for example, over 95 percent of adults are affected by untreated coronal decay and/or periodontitis, with the burden of disease concentrating in individuals of lower income and education, those who lack dental insurance, and those who decline recommended dental care because of costs. Such inequalities have led several national and international institutions, such as the Public Health Agency of Canada (PHAC), World Health Organization (WHO) and International Association for Dental Research (IADR), to call for a better understanding of the causal pathways in oral disease to inform public health policy and to guide new and innovative public health interventions (97).

From an etiological stance, PD has consistently been linked to the interplay between plaque and the host-immune response. Extensive research has shown that while periodontal conditions are initiated by dental plaque, perpetuation of inflammation and the severity and progression of the disease depends upon the effectiveness of the innate immune response to the bacterial biofilm (27, 98, 99). Meanwhile, dental caries is essentially a diet-mediated disease in which host factors, such as immune components in the microbial biofilm and saliva contribute to its progression (100, 101).

While several factors are known to modify variations in these host factors, such as genetic and systemic elements, they cannot explain, on their own, the social differences in oral health (102). Consequently, addressing these factors alone as the causes of oral disease
has resulted in reductionist approaches to prevention and treatment that often lack a sound theoretical basis, and that have generally been unsuccessful in reducing the burden of oral disease and oral health inequalities (103).

Similarly, considerable work to understand the causes of oral disease and related inequalities has focused on the role of behavioral factors such as poor oral hygiene, smoking and alcohol consumption, where poor oral health and related inequalities have been attributed to the lack of health education, especially in socially disadvantaged group (104, 105). However, downstream behavioural interventions (e.g. oral health education programs) have proven ineffective in achieving sustainable behavioural change (29, 30, 103, 106). In fact, studies suggest that such interventions have potentially widened the health gap between social groups (29). This failure has been attributed to ignoring the social, economic and psychosocial environments in which behaviours occur when designing such interventions (102).

Research in social epidemiology has drawn on the biopsychosocial pathway for plausible links between social factors and oral diseases. Evidence has consistently shown that chronic psychosocial stressors related to social adversity, such as low socioeconomic position (SEP), material deprivation and poor social relationships, can influence physiological body functioning, as well as behaviour in relation to disease (107). This has been characterized in studies demonstrating the social patterning of stress hormones and biomarkers in various health conditions such as cardiovascular disease (CVD), obesity, asthma and depression (48, 49, 89, 108-111).

Despite the association between adverse social conditions and health outcomes, a
lack of understanding persists as to the psychosocial and biological pathways through which the social becomes embodied, or ‘gets under the skin,’ to bring about disease and ultimately leading to health inequalities. Indeed, Marmot has suggested that deeming a particular psychosocial factor as both a cause of ill-health and a contributor to social inequalities in health requires an understanding of the biological mechanisms involved (35).

In this paper, we aim to understand the mechanisms by which social adversity becomes embodied to interact with biological systems in terms of oral disease. We first provide an overview of “embodiment” and then present a systematic review of the social-biological interplay in oral disease, with the purpose of identifying knowledge gaps in this area. Based on the results of our review, we propose a dynamic model that depicts the putative interdependencies between social and biological factors that lead to poor oral health. To conclude, we shed light on some of the public health policy implications and research opportunities in the field.

4.1.1 Understanding embodiment

Several studies have explained the social patterning in health by exploring mechanisms along the biopsychosocial pathway (45, 112-114). These suggest that health inequalities result from differences in the experience of stress between social groups due to material and psychosocial factors (115). For example, individuals of lower SEP experience higher levels of stress due to precarious living conditions and the inability to meet daily needs. Similarly, psychosocial factors such as lack of social support, job security and job strain, have been shown to relate to worse health outcomes (116-118). The resultant stress – both
over the life course and at different life stages – becomes embodied by altering the body’s neuroendocrine and immune responses, leading to an increased cumulative physiological burden and the initiation and progression of oral disease (117-119). Thus, in order to understand social-biological interactions and their promotion of poor oral health, it is fundamental that we grasp the pathophysiological mechanisms involved as well as the mediators by which stressors are transduced into the body.

4.1.2 The allostatic load experience

Perhaps one of the most compelling explanations of how social adversity translates into biological processes is that of “allostatic load” (60). Allostatic load represents the “wear and tear” on the body that results from repeated attempts to maintain homeostasis in response to prolonged stress challenges (120). When psychological demands exceed an individual’s capacity to adapt physiologically and emotionally, this can have physiological implications that may compound over time and trigger chronic disease (61). More specifically, the dysregulation of the hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal-medullary (SAM) axes produces stress hormones such as cortisol, epinephrine and norepinephrine, which in turn disrupt the neuroendocrine, immune and metabolic systems (63). This notion of stress acting as a common risk factor to the dysregulation of various body systems places an emphasis on the interaction between different biological systems in the body and considers the impact of cumulative physiological dysregulation over the life course as a disease risk (121). Indeed, allostatic load has been shown to increase the risk for a number of health conditions, including CVD, cognitive and physical decline and all-cause mortality (62-65).
A number of parameters have been used to measure allostatic load. McEwen proposed a summative score where markers such as systolic and diastolic blood pressure, waist-to-hip ratio, serum HDL and total cholesterol, glycated haemoglobin, and overnight urinary cortisol secretion can be used as an aggregate score (60, 66). These measures have been used with the aim of attaining a range of values for markers that include the different regulatory systems involved in adapting to allostatic load (66, 67). Interestingly, allostatic load has been suggested to have an additional predictive power in disease risk over individual biomarkers and provides a potential understanding of the physiological burden imposed by exposure to detrimental stressors.

Unsurprisingly, allostatic load is socially patterned, with higher allostatic load being associated with social disadvantage. Studies have shown that allostatic load is associated directly with senility, psychological stressors, poor neighbourhood conditions and low SEP (108, 122-124). Alternatively, healthy social ties as well as higher income and education levels seem to ameliorate the effects of allostatic load (122, 125). For instance, Kubzansky et al. linked SEP, measured by educational attainment, to psychosocial vulnerability and allostatic load, concluding that lower levels of education and greater levels of hostility are associated with higher allostatic load indices. Alternatively, higher income and education levels as well as healthy social ties have been shown to mitigate the effects of allostatic load (122, 125).

4.1.3 Behaviour as a mediator of stress

Allostatic load not only reflects the influence of social circumstances and stressful life experiences, but also behaviours, such as smoking, diet, exercise and alcohol consumption,
which influence the reactivity of physiologic systems to stress (120). These health behaviours are well-known common risk factors of several chronic health conditions such as CVD, obesity, diabetes, and some cancers, and have also been linked to all-cause mortality (126-131). However, it has been argued that their causal role in disease has been overstressed, and that they should be viewed as mediators of the adverse circumstances and psychosocial environment in which people live (132), instead of triggers of disease. Indeed, social and living conditions generating psychosocial stressors and material constraints determine whether individuals uptake harmful behaviour and whether they possess the necessary resources and motivation to care for their oral and overall health (37). Related to this is the link between the social environment and self-perceived health and health locus of control, which in turn affects one’s ability to change harmful behaviour (37). Therefore, while health is critically dependent on behaviour, it is necessary that behaviours are placed into context as mediators of the social, economic and psychosocial influences that are affected by stress and diminished coping abilities.

4.1.4 Immune responses to stress

With the growing realization that immune and inflammatory processes play a major role in a spectrum of chronic diseases (e.g. CVD, asthma, obesity, Alzheimer’s disease and PD) (133-137), the relationship between psychosocial stress and allostatic load, particularly in relation to immune system dysregulation, has become increasingly important. In this regard, studies have shown that stress hormones, including cortisol, epinephrine and norepinephrine, bind to specific leukocyte receptors and have deregulatory effects on their distribution and function (138). Large population-based studies have also shown that
leukocyte response to physiologic regulation by the HPA axis is altered under conditions of social isolation, eventually increasing the susceptibility to health risks. As well, chronic stress and lower SEP have been shown to suppress the phagocytic and bactericidal leukocyte functions, and to be associated with short-living leukocytes with shorter telomere lengths, a sign of cell senescence and premature ageing immunity (139, 140). In support of this concept, functional genomic analyses have found individuals with stress-induced anxiety to have a diminished expression of gene transcripts bearing the response elements for glucocorticoids while having up-regulated gene transcripts for pro-inflammatory transcription factors (94, 141). Genes for anti-inflammatory cytokines, such as IL-10 and IL-13 were also found to be overexpressed in response to stress (139). These findings of both pro and anti-inflammatory cytokine expressions associated with chronic stress response have been attributed to the repeated attempts of the body to counter-balance the effects of pro-inflammation by inducing anti-inflammatory responses (139), all pertaining to the “wear and tear” concept. These cascades would obviously predispose to increased and prolonged levels of systemic and possibly local inflammation.

4.1.5. Summary

Given all of the above, the relationship between social factors and immune processes in oral disease has emerged as an area of growing interest and study. Indeed, while the social patterning in oral disease has been attributed to differences in behavioural, social, economic and environmental factors, much remains underexplored in terms of how social exposures translate into biological processes. Despite the consensus that these factors are key determinants of oral disease, the psychosocial and biological pathways through which
social adversity undermines oral health remains unclear and is worth investigating. As a result, we undertook a systematic review, which is presented next, to examine the plausible relationships and interactions between the social and the biological in oral disease, and to define knowledge gaps and future research opportunities in this field.

4.2 Methods

4.2.1 Search strategy

We conducted a literature search to investigate the mechanisms linking social and psychosocial exposures to biological markers in oral disease. Published articles were obtained by searching databases, including Ovid MEDLINE, Embase, Web of Science and PsycINFO. We used the past two decades as a timeframe for our literature search (1994-2015). This timeframe was guided by the five eras of health inequalities research proposed by Adler and Stewart, where research into the underlying biological mechanisms of health inequalities started in the early 2000s (115). The search used appropriate keywords and MESH terms (exploded) of the exposure and outcome of interest (Table 4-1). The search was complemented by screening the references of selected articles for studies that did not appear in the database search.

4.2.2 Study selection

To be included in this review, a study had to assess two aspects in relation to oral health outcomes: a psychosocial stressor and at least one biological marker. Studies were excluded if assessing acute, time-limited stressors (e.g. students taking examinations), physical stressors (e.g. cold, physical restraint) and if the oral health condition studied was
acute, transient or self-limiting (e.g. dental trauma, recurrent herpes simplex, minor aphthous ulcers). Only articles published in the English language were considered, and no restrictions were made on the age group studied. Although a chronic oral condition, oral cancer was not the focus of this study due to the number of factors on the biological front related to its pathogenesis, which may be different than those of other oral disease generally. Finally, titles and abstracts from the initial search were reviewed to select potentially relevant articles for full review.

4.2.3 Data extraction and quality assessment

Quality assessment of the studies was carried out using the National Institute of Health (NIH) quality assessment tool for observational studies as applicable. Due to the heterogeneity of the methodologies and study designs, and as the purpose of this work was to identify potential mechanisms of social-biological interactions rather than evaluating the strength of the evidence, quantitative evaluation of the studies was not possible. Therefore, the results are presented in the narrative form.

4.3 Results

4.3.1 Study characteristics

A total of ten studies were eligible for inclusion in this review, summarized in Table 4-2. The studies had different designs: four cross-sectional, four case-control and two longitudinal studies with 6-months and 5-years of follow-up, respectively. There was considerable variation amongst the studies as per population characteristics, psychosocial conditions of interest, methods of stress assessment and the covariates taken into account.
Most studies investigated the relationship between psychosocial stress and PD and included a broad range of age groups (18-85 years). Dental caries in relation to psychosocial exposures was examined in two studies (6, 142). Data from epidemiological surveys were used in four studies (69, 70, 142-144), whereas others recruited participants from university periodontology clinics (145), private practices (146), or through files of employees on sick leave (147). The quality of the studies is described in Table 4-3.

4.3.2 Oral health outcomes

Parameters used to assess PD varied between studies. In most studies, PD was assessed by measuring periodontal health parameters, including probing depth, clinical attachment loss, bleeding on probing and plaque and gingival indices (6, 69, 70, 143-146, 148, 149). Dental caries was measured through decayed, missing and filled teeth/surfaces in primary and permanent teeth (142).
Table 4-1. Keywords, MESH terms, inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Social, socioeconomic, psychosocial. Keywords: stress, psychosocial, psychosocial stress, psychological stress, socioeconomic status, income, education</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome</td>
<td>Oral disease, periodontitis, dental caries, changes in biological markers. Keywords: oral health, oral disease, periodont*, dental caries, tooth loss, immun*, biological markers, oral health inequalities, oral health disparities</td>
</tr>
<tr>
<td>Study design</td>
<td>No restrictions on study-designs were applied</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Empirical studies, English language, human studies</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>Non-empirical studies</td>
</tr>
</tbody>
</table>
Table 4-2. Summary of key studies exploring the relationship between structural/social, psychosocial and biological factors in oral disease

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study design</th>
<th>Oral health outcome</th>
<th>Population</th>
<th>Structural/social factors</th>
<th>Psychosocial factors</th>
<th>Behavioural factors</th>
<th>Biological marker(s)</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moss et al. (143)</td>
<td>Case-control</td>
<td>Periodontitis (plaque, AL, BOP, PRD)</td>
<td>Participants from Erie County Risk Factor Study (71 cases, 77 controls), U.S</td>
<td>-</td>
<td>Daily Strains (job strain, financial strain, spouse strain, strain related to parenting children); psychological distress and coping style. Strain Scale; Brief Symptom Inventory; COPE inventory</td>
<td>Smoking</td>
<td>Antibody titres for periodontal pathogens</td>
<td>Depression as marker for social isolation was associated with elevated levels of antibody titres for PRD at baseline and after 1-year follow-up</td>
</tr>
<tr>
<td>Giannopoulou et al. (148)</td>
<td>Case-control</td>
<td>Gingivitis, AP, EOP (PI, BOP, SUP)</td>
<td>Participants were selected from a private practice limited to periodontics in Athens, Greece (80)</td>
<td>-</td>
<td>Perceived stress, Modified and Perceived Stress Scale (MAPS)</td>
<td>Smoking</td>
<td>IL-1b, IL-4, IL-6 and IL-8 in the GCF</td>
<td>IL-4, IL-6 and IL-8 were significantly correlated with to smoking while stress was associated with IL-1b, IL-6 and IL-8 levels</td>
</tr>
<tr>
<td>Mengel et al. (145)</td>
<td>Case-control</td>
<td>AGP, ALP, CGP (GI, PI, CAL)</td>
<td>Patients from periodontology department, Philips-University, Marburg, Germany (40 cases; 40 controls)</td>
<td>-</td>
<td>Job-related stress, family-related stress, attitude to life. Questionnaire (non-validated)</td>
<td>Smoking</td>
<td>IL-1β, IL-6, cortisol in serum</td>
<td>No correlation between immunological markers, cortisol and the registered stress values. Patients with untreated AGP showed a pessimistic attitude to life and elevated serum IL-6. Sample size was too small for generalizable conclusions. Method of stress assessment was unvalidated and unstandardized</td>
</tr>
<tr>
<td>Johannsen et al. (150)</td>
<td>Case-control</td>
<td>Periodontitis (plaque, GI, CAL, PD); Number of teeth</td>
<td>Women on long-term sick leave for depression (20 cases, 29 controls), Stockholm, Sweden</td>
<td>-</td>
<td>Job-stress related depression (DSM-IV)</td>
<td>Smoking</td>
<td>IL-1β, IL-6, MMP-8, MMP-9</td>
<td>Women on long-term sick leave for depression had more plaque accumulation and higher concentrations of GCF IL-6 than controls, suggesting relationships between depressive symptoms and immune changes</td>
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<td>Author et al.</td>
<td>Design</td>
<td>Periodontitis (CAL, GB)</td>
<td>Study (Years)</td>
<td>SEP (PIR; education)</td>
<td>Smoking</td>
<td>Allostatic load</td>
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<tr>
<td>Borrell et al. [70]</td>
<td>Cross-sectional</td>
<td>NHANES (1999-2004), United States</td>
<td>SEP (annual family income, PIR)</td>
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<td></td>
<td>Allostatic load increases the probability of periodontitis. This association is explained by race/ethnicity.</td>
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<tr>
<td>Boyce et al. [6]</td>
<td>Cross-sectional</td>
<td>Kindergarten children from the Peers and Wellness Study (n=94), East San Francisco Bay Area, California, U.S</td>
<td>Socioeconomic status (parent-reported highest household education level)</td>
<td>Family financial stressors (FSS)</td>
<td></td>
<td>Low SES, higher basal salivary cortisol and larger numbers of cariogenic bacteria were each significantly and independently associated with caries. Higher salivary cortisol reactivity was associated with thinner, softer enamel surfaces in exfoliated teeth. Highest rates of dental pathology were found among children with the combination of elevated salivary cortisol expression and high counts of cariogenic bacteria.</td>
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<td>Bakri et al. [146]</td>
<td>Longitudinal (6 months follow-up)</td>
<td>Periodontitis (PRD, BOP)</td>
<td>45 patients with periodontitis in need of NPT, Sheffield, UK</td>
<td></td>
<td>Perceived stress (PSS)</td>
<td>Smoking</td>
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<tr>
<td>Buchwald et al. [144]</td>
<td>Longitudinal (5-year follow-up)</td>
<td>Periodontitis (AL); Number of teeth</td>
<td>3300 participants from Study of Health in Pomerania (SHIP), Germany</td>
<td>Socioeconomic status (education, occupation, household income), marital status</td>
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<td>Patients under psychosocial stress had increased elastase levels and poorer outcomes following NPT</td>
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Masterson et al (142) | Cross-sectional | Caries in children | 1184 mother-child pairs using NHANES III | PIR | - | Maternal care-taking behaviours | Maternal allostatic load | Maternal allostatic load is associated with caries in children and is linked to health-related maternal behaviours
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<td>Was the research question or objective in this paper clearly stated and appropriate?</td>
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<td>Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?</td>
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<td>If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?</td>
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1=Yes; 0_No; NA=not applicable, NR=not reported
4.3.3 Mechanisms of social-biological interactions in oral disease

4.3.3.1 SEP, allostatic load, and susceptibility to inflammation

Using American NHANES data, two epidemiological studies by Sabbah et al. and Borrell et al. examined the relationship between SEP (poverty: income), allostatic load and PD. (69, 70) Allostatic load was summed using measures of central obesity, blood pressure, hypertriglyceridemia, low HDL, plasma glucose, C-reactive protein (CRP) (a marker of systemic and local inflammation) and fibrinogen. Sabbah et al. found allostatic load to explain the social gradient in both PD and ischaemic heart disease. Meanwhile, Borrell et al. found the relationship between allostatic load and periodontitis to vary by race/ethnicity and attributed that to the stronger effect allostatic load may play in racial minorities who may lack coping strategies. The significance of allostatic load as outlined earlier in this review, lies in the cumulative effects of psychosocial stress that can be detrimental to different body systems, acting as a common pathway to several health conditions, including oral disease. Moreover, these effects seem to extend beyond influencing only one’s oral health. For example, in a recent study by Masterson and Sabbah, maternal allostatic load – as a measure of exposure to chronic stress – was linked to adverse care-taking behaviours, such as breast-feeding, taking a child to a dentist, and giving them breakfast daily, and was correlated to child caries experience (142). These associations were attenuated after adjusting for SEP. While these behaviours have not been conclusively related to oral disease in children, the study highlights how the cumulative effect of chronic stress, as a result of poor living conditions, can extend to affect maternal behaviours and ultimately their children’s health.
In a longitudinal epidemiological study, Buchwald et al. found the combination of SEP (income and education) and behavioural factors (smoking) to augment the progression of PD and to be associated with higher levels of obesity and CRP. They suggested that low SEP is associated with an increased susceptibility to inflammation, and that inflammatory challenges (high levels of CRP, obesity) are most adverse in individuals with low SEP (144). An important highlight of this study was the gender difference in disease and inflammation. Women had better periodontal health than men, in accordance with previous studies showing that women take better care of their health and smoke less than men (151). However, while women had higher grades of education, they earned less than their male counterparts and also had higher levels of CRP, indicating an increased susceptibility to inflammation. (144) Although this study did not include psychosocial assessments, it could be hypothesized that gender inequalities may be a potential psychosocial stressor that predisposes subjects to an increased risk of inflammation.

4.3.3.2 SEP, cortisol and pathogenic bacterial load

Boyce et al. studied the role of social disadvantage in childhood caries by assessing SEP, family financial stressors, salivary cortisol levels and the number of cariogenic bacteria in relation to dental carious lesions (6). The study showed that low SEP, basal activation of children’s HPA axis, and higher counts of cariogenic bacteria were each significantly and independently associated with higher risk of dental caries. In fact, the highest rates of disease were exhibited amongst children with a combination of both elevated salivary cortisol levels and cariogenic bacteria. This can be explained through studies showing the ability of stress to impair sIgA production (an important player in the regulation of oral
microflora) and to suppress immunity and inhibit immune cell aggregation through the release of endogenous glucocorticoids (152).

Thus, the role of stress and cortisol in contributing to an increased cariogenic bacterial load and hence the susceptibility to dental caries becomes plausible. In fact, this mechanism also applies to PD and is in accordance with previous observations by Moss et al., in which they reported increased levels of antibody titres to periodontal pathogens (*Bacteroides forsythus*) in periodontitis patients who had symptoms of depression and who experienced social isolation. Furthermore, Boyce et al. also found higher salivary cortisol reactivity to be associated with thinner, softer enamel surfaces in exfoliated teeth in children in relation to their race/ethnicity.

Previous studies have shown that glucocorticoids can induce enamel hypoplasia in children (153), affect new dentin formation and the size of the pulp chamber in animal models (154), ultimately contributing to an increased susceptibility to dental caries. Importantly, the above observations give insight into understanding oral disease over the life-course by showing that social disadvantage can interact with biological processes to determine biological inequalities that can have long-term effects, such as the physical properties of teeth and the cariogenic bacterial load. These are eventually carried into adulthood, resulting in a long-lasting, chronic disease burden and a further contribution to inequalities in oral health.

### 4.3.3 Stress, depression and inflammatory biomarkers

Giannopoulou et al. reported on the relationship between gingival crevicular fluid (GCF) interleukins with stress in periodontitis, where perceived stress was associated with
increased levels of IL-1β, IL-6 and IL-8 (148). It has been suggested that the high levels of cytokines in response to stress may be explained by the alteration in Th1/Th2 balance due to cortisol release, which results in a shift towards a pattern of humoral response and cytokine production by Th2 cells (155). In a longitudinal study, Bakri et al. investigated the effects of perceived stress on the outcome of non-surgical periodontal treatment, and on markers of periodontal inflammation and bone destruction, including GCF neutrophil elastase and C-terminal telopeptide of type I collagen (146). Their results showed that stressed patients had significantly higher elastase levels and poorer treatment outcomes following non-surgical periodontal therapy compared to the non-stressed.

An important aspect of the relationship between perceived stress and inflammatory biomarkers is understanding that stress is not the same experience for everyone and depends on a number of psychosocial factors such as coping abilities, social support and the quality of social networks (102). In addition, one’s self-perception of stress may also relate to factors such as health behaviours, and the belief of internal versus external locus of control (102). Thus, investigating how these factors may interact and contribute to the inflammatory process is important.

Johannsen et al. investigated periodontal status in relation to interleukins and cortisol in GCF and saliva of women on long-term sick leave for depression (150). The study found depressed women to have significantly higher levels of PD and GCF IL-6 after adjusting for age and smoking. These observations are supported by previous work that shows an association between depression and social isolation and levels of Tannerella forsythia in the plaque of PD patients. Meanwhile, individuals suffering depression have also been shown to be more prone to refractory periodontitis, a condition of continued
disease progression and clinical attachment loss that does not correlate with plaque levels, microbiological assessments or treatment compliance, and in which hyperactive neutrophils (cells of the innate immune system) have been shown to play an important role (156, 157). With recent studies suggesting depression to be an inflammatory condition characterized by activated cell-mediated immunity and immune cell glucocorticoid resistance (158, 159), again, a possible common stress pathway can potentially be at play in the pathogenesis of both conditions.

4.3.3.4 Health behaviours

A number of studies included in this review have shown that the relationship between social and psychosocial factors, inflammatory biomarkers and PD can be explained by smoking, indicating that behaviour is a link through which social and psychosocial conditions can lead to oral disease. This corresponds to studies that have shown behaviours to partly explain the social gradient in oral health (160), and goes with the notion that behaviours are psychosocial in nature in the sense that they are responses to psychosocial stressors and adverse circumstances, and should therefore be considered as mediators of oral disease.

Yet a few studies have found health behaviours (e.g. smoking) to not explain the relationship between SEP and inflammatory biomarkers or depression and inflammation (144)(150), This suggests that, behaviours, although considered as mediators of stress, are not the only pathway through which stress translates into disease, further suggesting a direct pathway through which social factors can affect biological processes, independent of health behaviours.
4.4 Conceptualizing social-biological interactions in oral disease

Despite the plethora of evidence on the social and psychosocial influences on oral health outcomes, only a few empirical studies were found that have considered the social causes of stress and their potential effects on the pathophysiology of oral disease. Some difficulty was encountered in arriving at the specific mechanism(s) of social-biological interactions due to a number of factors. First, is the complex nature of the topic and the multiple variates and co-variates that may be at play in the development and progression of oral disease. To be sure, the populations examined in the studies varied from groups in population surveys to individuals recruited at university clinics. Different types of social stressors where studied varying between job-stress, family stress and financial stress. Also, the method of stress assessment varied between psychometric and biochemical methods. This diversity made it difficult to compare results between studies. However, some putative mechanisms of social-biological interactions can be abstracted from the summative results of this review, which we have plotted as a dynamic conceptual model of the plausible social-biological interactions (Figure 4-1). In addition, some links in the model are derived from the literature on the social determinants of oral and general health, and have not been included in this review.

Conceptualizing the social causes of oral disease has been robustly discussed in the literature with several theoretical explanations, varying in their emphasis and standpoint, ranging from materialist, behavioural, psychosocial and life-course perspectives (34, 96, 161, 162). Several mega-models have depicted the relationships between social factors and health, and have provided the theoretical basis for much of the work on the social
determinants of oral and general health (16, 37, 39, 163, 164). These models initiated a paradigm of integration between the social and biological determinants of health, elaborating on how biological pathways exist in a social and psychosocial context. Indeed, the models not only provide an understanding of how potential causal factors may interrelate, but also serve as guides to points of intervention. However, many of these models depict the social factors as distal antecedents to the more proximal biological causes of disease and do not elaborate on the complexity of the dynamic and reciprocal relationships that potentially exist between social and biological factors. In spite of their contribution to policy-making, models that conceptualize social factors as antecedents to biological factors without considering the multiple interactions that may exist between them may have created and/or promoted a false dichotomy between the social and the biological, and may have indirectly given biomedical researchers and clinicians the notion that it may be possible to ignore the social factors and intervene only at the level of the proximal causes of disease. Thus, despite repeated calls to incorporate social causes into health research, social antecedents models might not change how biomedical research is conducted and how biological questions are framed and answered (26).

Therefore, understanding social-biological interactions in oral disease requires a view of the social as intertwined with the biological. Here, we propose a conceptual model that illustrates the dynamicity and reciprocity between the different social and biological factors in oral disease, and that shows disease as the result of complex interactions between molecular, biological and social systems with positive and negative feedback loops (25).

Dynamic models depicting such complex relationships have been previously applied to epidemiology to understand the mechanisms of disease including work on
diabetes and CVD, and have been employed in understanding cellular and molecular pathways of disease (165-167). Our model demonstrates the potential putative interdependencies between the different factors involved in oral disease, illustrating that psychosocial stress results from and affects socioeconomic conditions and the social and work environment, eventually leading to behavioural and biological changes that compromise the body’s protective mechanisms against oral disease. Under this model of social-biological interactions, social and psychosocial experiences have the capacity to directly alter both the structure and function of biological systems. Thus, these factors are not viewed as distinct and susceptible to separation but rather as integral parts of a complex system that ranges from epigenetic and cellular reactions to structural factors, where the social environment is a key piece in understanding the function of the system (26). The dynamic loops in the model facilitate deriving empirically testable relationships. It is important to note, however, that the purpose of the model is not to include all geopolitical and structural determinants of oral health, but to provide an understanding of the possible interactive relationships between social adversity and pathobiological processes in oral disease. Therefore, more variables, relationships and dynamic loops can be added onto the model as interdisciplinary research in this area advances.
Figure 4-1. Dynamic conceptual model of social-biological interactions. The model demonstrates the interdependent relationships between different variables involved in oral disease. Blue lines are relationships derived from studies in Table 2. Orange lines represent hypothetical relationships. (R): reinforcing loops; signs (+/-) on arrowheads: polarity of the relationship between variables.
4.5 Policy implications and research opportunities

People live in political, social and economic systems that shape their access to resources, behaviour and biology. Understanding the pathways through which these factors interplay to shape oral health inequalities can help inform public health policies about the causal pathways to oral disease and can therefore lead to a much needed paradigm shift towards public health interventions at the level of the fundamental conditions that put people at “risk of risks” – as described by Link and Phelan (36). However, unraveling these pathways requires refuting the existing false dichotomies between the social and the biological, and developing research approaches that give equal weight to both the social on the one hand, and the biological on the other.

In a critical review of theories in social epidemiology, Krieger suggests that theories and research approaches are needed that recast differences in biological outcomes as embodied expressions of modifiable social exposures.(168) In that regard, research on the social-biological interactions in oral disease represents a fertile field not only to identify social and biological risk factors, but also to recognize the interplay between social exposure, susceptibility and resistance. Ultimately, this can help identify those at higher risk for developing oral disease due to adverse social circumstances, and prevent and/or control such disease experience.

While addressing the social and living conditions in which we live has arguably been hindered by weak policies, bias to downstream approaches and a lack of political will, understanding the biology of social adversity and identifying how social and material factors become incorporated within biology is integral to developing evidence-informed
arguments on the social origin of disease. This is particularly important if we are to devise new and novel interventions that address the social determinants of health and tackle oral health inequalities.
Chapter 5

Study II: How Does the Social “Get Under the Gum”: The Role of Socioeconomic Position in the Oral-Systemic Health Link

This study was published in the Canadian Journal of Public Health:


Abstract

Objectives: To evaluate the extent of association between systemic inflammation and PD in American adults, and to assess whether socioeconomic position (SEP) explained this relationship.

Methods: We used data from the National Health and Nutrition Examination Survey (NHANES IV) (2001-2010). Systemic inflammation was defined by individual and aggregate (cumulative inflammatory load) biomarkers (C-reactive protein, white blood cell counts, neutrophil counts and neutrophil: lymphocyte ratio). Loss of attachment and bleeding on probing were used to define PD. Poverty: income ratio and education were indicators of SEP. Covariates included age, sex, ethnicity, smoking, alcohol, and
attendance for dental treatment. Univariate and multivariable logistic regressions were constructed to assess the relationships of interest.

**Results:** In a total of 2,296 respondents, biomarkers of systemic inflammation and cumulative inflammatory load were significantly associated with PD after adjusting for age, sex and behavioural factors. SEP attenuated the association between markers of systemic inflammation and PD in the fully adjusted model.

**Conclusion:** SEP partly explains how systemic inflammation and PD are coupled, and may thus have a significant role in the mechanisms linking oral and non-oral health conditions. It is of critical importance that the social and living conditions are taken into account when considering prevention and treatment strategies for inflammatory diseases, given what appears to be their impactful effect on disease processes.
5.1 Introduction

The oral-systemic health link continues to emerge as an area of new interest and study from clinical and public health policy standpoints (169). This stems largely from this field’s significant implications in advancing knowledge on the biological processes linking these groups of diseases, and in innovating strategies for their diagnosis, prevention and treatment. Yet, many of the underlying pathways between oral and systemic conditions remain unknown.

Mounting evidence has shown a bidirectional relationship between PD and several other health conditions, with evidence suggesting that inflammation plays a pivotal role in these relationships (170-172). However, the risk factors involved in these inflammatory reactions are yet to be elucidated. Indeed, devising novel disease prevention strategies requires identifying these risk factors and understanding how they affect the disease process. For example, oral health policy researchers have suggested that effective disease prevention should target risk factors that are common to both oral and systemic diseases, including health-related behaviours and health-system factors (34). Nonetheless, these risk factors have been continuously critiqued for their limited ability in explaining oral and non-oral diseases and related health inequalities (34, 160). Meanwhile, the “fundamental causes” of disease are continuously argued to lie within the socio-political factors as the key determinants of health (34, 160, 173). Indeed, this conforms with the general susceptibility view of disease causation, which suggests that there are common, rather than specific risk factors that affect people’s vulnerability and susceptibility to a wide range of chronic conditions (174).
Previous research has proposed that disease is not merely the product of cellular and molecular cascades taking place within the body, but rather the result of an interplay between social factors, such as SEP and biological ones, such as the immune system (175, 176). These were suggested to interact through various reciprocating pathways, eventually leading to an increased risk of either or both oral and systemic conditions diseases (175).

Given the growing interest in the role of social and living conditions in bringing about disease - most notably, the impact of socioeconomic conditions on the mechanisms of inflammation - the need to understand the interplay between social and biological factors has continued to gain importance and is now being brought to the forefront of clinical and public health research.

In this study, we aimed to investigate the role of SEP as a structural factor in linking oral and systemic inflammation. Using a nationally representative sample of American adults, we assessed the extent of association between systemic and periodontal inflammation, and whether socioeconomic factors mediated this relationship.

5.2 Methods

5.2.1 Study design

Data for this research were publicly available and obtained from the Continuous National Health and Nutrition Examination Survey (NHANES IV). NHANES uses a stratified multistage probability sampling design with a sample representative of the non-institutionalized American population. The survey collects a variety of cross-sectional data
via questionnaires, physical examinations and laboratory assessments. For this study, we
used NHANES IV cycles that spanned the years from 2001 to 2004 and 2009-2010, for
adults aged 20-85 years who had received a clinical periodontal examination and laboratory
assessments. Cycles 2005-2006 and 2007-2008 did not include periodontal examinations
and were therefore excluded. Individual participants were excluded if they had reported
having diabetes or a cardiovascular condition, or if they had recently experienced an
episode of acute infection (e.g. sore throat, common cold), which could contribute to
increased inflammation.

5.2.2 Variables

5.2.2.1 Biomarkers of systemic inflammation

Systemic markers of inflammation included C-reactive protein, white blood cell counts,
segmented neutrophil counts, and neutrophil: lymphocyte ratio, obtained from laboratory
data of C-reactive protein assays and complete blood counts. The choice of these
biomarkers was based on their significant role in both periodontal and systemic
inflammation. C-reactive protein is an acute phase protein (a direct and quantitative
measure of the acute phase reaction) that reflects local and systemic events accompanying
inflammatory responses (e.g. vasodilatation, platelet aggregation, neutrophil chemotaxis,
and release of lysosomal enzymes). It is associated with several inflammatory conditions,
including PD. Total white blood cell counts are also a measure of systemic inflammation
and were suggested to mediate the relationship between systemic and periodontal
inflammation (177). Derived from the differential white blood cell count, neutrophils are
cells of the innate immune system that play a crucial role in systemic inflammation and PD,
and which have recently been employed in understanding the oral-systemic health links (178, 179). Neutrophil: lymphocyte ratio has also been used in several studies as an index of systemic inflammation and as a prognostic factor for a number of diseases (180, 181). In addition to these four biomarkers, we created a clustered dichotomous variable for cumulative inflammatory load based on the American Association for Clinical Chemistry cut-off values for neutrophils (7.5 x 10^9 cells/L) and white blood cells (11.0 x 10^9 cells/L) (182).

5.2.2.2 PD assessment

The NHANES periodontal examination is performed according to a random half-mouth method (excluding third molars). Pocket depths (PRD), clinical attachment loss (AL) and bleeding on probing (BOP) measurements were assessed at three sites per tooth (mid-facial, mesial and distal). For the 2009-2010 cycle, a full-mouth examination was performed using six sites per tooth. To standardize measurements across NHANES cycles, we first created variables to indicate the extent of PD. These were the extent of P≥4mm, the extent of AL≥3mm and the extent of BOP, each calculated as the ratio of sites showing the periodontal characteristic to the total number of sites probed. We then created a binary variable for periodontal disease as having at least one percent of sites with BOP and P≥4mm, or one percent of sites with BOP and AL≥3mm. These PD indicators were similar to ones used in previous NHANES studies (18, 183).

5.2.2.3 Risk factor assessment

Poverty: income ratio and years of education were used as indicators of SEP. Poverty: income ratio was calculated in NHANES by dividing family income by the poverty
thresholds, specific to family size, as well as the appropriate year and State according to the
American Department of Health and Human Services guidelines. If family income was
reported as a range value, the midpoint of the range was used to compute the variable.
Poverty: income ratio values in NHANES range from 0 to 5, with the cut-off of 1
indicating an individual or family as below the poverty threshold. Education was defined as
the highest grade or level of education completed. We dichotomized this variable into less
than 12 years (did not complete high school) and more than 12 years of education
(completed high school). Other variables included age, sex, ethnicity and behavioural
factors (smoking status, amount of alcohol consumption, dental attendance).

5.2.3 Statistical analysis

NHANES uses a complex, multistage, probability sampling design to select participants
that are representative of the civilian, non-institutionalized U.S. population. While
oversampling of certain population subgroups is generally done in national surveys to
increase the reliability and precision of health status indicator estimates for these groups,
sample weights are used to produce an unbiased national estimate (184). Here, we used
NHANES-constructed sample weights to take into account survey non-response, over-
sampling, post-stratification, and sampling error, and accordingly used survey commands
in STATA IC 14.1 throughout the analysis. Descriptive statistics were calculated. Testing
for the confounding effect of variables was carried out using a backward stepwise process
with a cut-off value of 10% change in the coefficient of PD. Using univariate logistic
regression, we first examined the crude association between the individual and aggregate
markers of systemic inflammation and PD. We then constructed multivariable logistic
regression models to assess the extent of association between systemic inflammation and PD while accounting for the effects of demographic, behavioural and socioeconomic factors. For this, we first adjusted the model for age, sex and behavioural factors (smoking, alcohol and dental attendance). SEP indicators were then added to the model to test for their effect.

5.3 Results

5.3.1 General characteristics

A total of 2,296 respondents were included in this study, after excluding those ineligible for participation. The mean age of respondents was 46.02 ± 16.6 (mean ± SD), with 62% being male (Table 5-1). Whites, Hispanics and Blacks represented 56%, 25% and 16% of the sample, respectively. Almost 72% had completed a high school education and were above the poverty threshold with a mean poverty: income ratio of 2.8 ± 1.6 (mean ± SD). Just over 20% of the participants had a total annual household income of less than $20,000, whereas 23% had an annual household income of more than $75,000. PD was observed in 25% of the sample participants. White blood cells, neutrophils and C-reactive protein were shown to have a mean ± SD of 7.29 ± 2.16 (x10⁹ cells/L), 4.31 ± 1.72 (x10⁹ cells/L), and 0.43 ± 0.93 mg/dL. Only 7% of the sample was shown to have above threshold cumulative inflammatory load.

An increased risk of PD was observed in males, and in association with age, as well as with behavioural factors such as smoking (OR=1.66, 95%CI 1.11-2.49) alcohol consumption (OR=1.44, 95%CI 1.10-1.88). Meanwhile, dental attendance was associated with lower PD
risk (OR=0.5, 95%CI 0.3-0.6). No significant differences were observed in PD risk when comparing ethnic groups.

5.3.2 SEP associates with systemic inflammation and PD

SEP had a protective association with PD. One unit increase in poverty: income ratio and obtaining more than 12 years of education reduced PD risk by 13% and 59%, respectively. Similarly, the risk of systemic inflammation was shown to be significantly reduced with higher SEP. Individuals with higher education had less risk of systemic inflammation as demonstrated by all individual and aggregate biomarkers, with the exception of neutrophil: lymphocyte ratio. Higher poverty: income ratio was associated with reduced C-reactive protein levels (Table 5-2).

5.3.3 Effect of SEP on the association between systemic inflammation and PD

Crude analysis showed individual and aggregate markers of systemic inflammation to be significantly associated with PD. For example, a unit increase in white blood cells, neutrophil counts and cumulative inflammatory load was shown to increase PD risk by 3.3, 2.1 and 2 times, respectively (Table 5-3, Model 1). A greater extent of loss of periodontal attachment, pocket depth and bleeding from probing were also shown to associate with increased white blood cells and neutrophils in the unadjusted models. Meanwhile, C-reactive protein was only associated with the loss of periodontal attachment. Adjusting for age, sex, ethnicity and behavioural factors in multivariable logistic regressions did not attenuate these relationships (Table 6, Model 2). In the fully adjusted model, the effects of
poverty: income ratio and education were demonstrated by the attenuation of cumulative inflammatory load and C-reactive protein. While blood cell counts remained were also partly attenuated by adjusting for SEP (Table 5-3, Model 3) (Figure 5-1).
Table 5-1. General characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristics (n= 2,296)</th>
<th>n (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46 ± 16.6</td>
<td></td>
</tr>
<tr>
<td>Sex (males)</td>
<td>1,510 (61.9)</td>
<td></td>
</tr>
<tr>
<td>Annual household income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; $10,000</td>
<td>146 (6.5)</td>
<td></td>
</tr>
<tr>
<td>10,000 – 19,999</td>
<td>314 (14)</td>
<td></td>
</tr>
<tr>
<td>20,000 – 34,999</td>
<td>464 (20.7)</td>
<td></td>
</tr>
<tr>
<td>35,000 – 54,999</td>
<td>512 (22.8)</td>
<td></td>
</tr>
<tr>
<td>55,000 – 74,999</td>
<td>290 (12.9)</td>
<td></td>
</tr>
<tr>
<td>&gt; $75,000</td>
<td>512 (22.8)</td>
<td></td>
</tr>
<tr>
<td>Poverty: income ratio</td>
<td>2.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12 years (no high school)</td>
<td>672 (27.5)</td>
<td></td>
</tr>
<tr>
<td>≥ 12 years (completed high school)</td>
<td>1,766 (72.4)</td>
<td></td>
</tr>
<tr>
<td>PD (binary)</td>
<td>602 (24.6)</td>
<td></td>
</tr>
<tr>
<td>Extent of pocket depth (mm)</td>
<td>3.3 ± 9.8</td>
<td></td>
</tr>
<tr>
<td>Extent of bleeding from probing</td>
<td>11.8 ± 18.1</td>
<td></td>
</tr>
<tr>
<td>Extent of loss of attachment (mm)</td>
<td>10.1 ± 20.6</td>
<td></td>
</tr>
<tr>
<td>Neutrophil count (x10^9 cells/L)</td>
<td>4.3 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>White blood cell counts (x10^9 cells/L)</td>
<td>7.2 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Neutrophil: lymphocyte ratio</td>
<td>2.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Cumulative inflammatory load</td>
<td>208 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Smoking Status (smokers)</td>
<td>1,266 (51.9)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption*</td>
<td>1,114 (45.6)</td>
<td></td>
</tr>
<tr>
<td>Dental attendance§</td>
<td>42.8</td>
<td></td>
</tr>
</tbody>
</table>

* At least one episode of having more than 3 drinks per day in the past year
§ Visited the dentist at least once in the past six months
Table 5-2. Association between indicators of SEP and biomarkers of systemic inflammation and PD

<table>
<thead>
<tr>
<th></th>
<th>Education OR (95% CI)</th>
<th>Poverty: income ratio OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>0.4 (0.2-0.6)*</td>
<td>0.8 (0.7-0.9)*</td>
</tr>
<tr>
<td>Systemic inflammatory markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>0.3 (0.1-0.7)**</td>
<td>0.3 (0.1-0.8)*</td>
</tr>
<tr>
<td>PMN</td>
<td>0.5 (0.2-0.9)*</td>
<td>0.6 (0.3-1.3)¶</td>
</tr>
<tr>
<td>NLR</td>
<td>0.8 (0.7-1.0)¶</td>
<td>1.0 (0.7-1.3)¶</td>
</tr>
<tr>
<td>CRP</td>
<td>0.4 (0.2-0.7)*</td>
<td>0.38 (0.1-0.7)*</td>
</tr>
<tr>
<td>CIL</td>
<td>0.6 (0.4-0.9)*</td>
<td>0.7 (0.4-1.3)¶</td>
</tr>
</tbody>
</table>

WBC: white blood cells; PMN: neutrophils; NLR: neutrophil: lymphocyte ratio; CRP: C-reactive protein; CIL: cumulative inflammatory load. *** p<0.001, ** p< 0.01, *p<0.05, ¤not significant.
Table 5-3. The effect of SEP on the association between systemic inflammation and PD

<table>
<thead>
<tr>
<th>Variables</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1 OR (95% CI)</td>
</tr>
<tr>
<td>WBC</td>
<td>3.3 (1.7, 6.3)***</td>
</tr>
<tr>
<td>PMN</td>
<td>2.1 (1.2, 3.6)***</td>
</tr>
<tr>
<td>NLR</td>
<td>1.2 (0.7, 2.1)†</td>
</tr>
<tr>
<td>CRP</td>
<td>1.1 (1.0, 1.3)*</td>
</tr>
<tr>
<td>CIL</td>
<td>2.0 (1.0, 3.9)*</td>
</tr>
</tbody>
</table>

PD: periodontal disease; WBC: white blood cells; PMN: polymorphonuclear leukocytes; NLR: neutrophil to lymphocyte ratio; CRP: C-reactive protein; CIL: cumulative inflammatory load. **p<0.01, *p<0.05, †not significant.
Figure 5-1. SEP partly attenuates the association between systemic inflammation and PD. Odds ratios (95% CI) of PD in association with systemic inflammatory markers are shown for the crude model, partially adjusted model (for age, sex, ethnicity and behaviour) and the fully adjusted for SEP.
5.4 Discussion

In this study, we found PD risk to be significantly associated with higher levels of systemic inflammation, supporting previous studies that demonstrated a relationship between various inflammatory conditions and PD (185-187). This association remained statistically significant after controlling for factors known to trigger both, systemic and periodontal inflammation such as smoking and alcohol consumption and independent inflammatory conditions such as cardiovascular disease and diabetes.

In addition, the systemic inflammation-PD association was partly attenuated by SEP, indicating its role in the inflammatory processes linking oral and systemic diseases; a role that may outweigh health behaviours. Previous research has highlighted the ability of social conditions to “get under the skin” to affect one’s biological systems and hence the body’s ability to fend off disease (111, 175, 176). The effect of SEP observed in this analysis supports this concept, where social and economic conditions that determine an individual’s position on the social hierarchy can potentially influence immune functions – indicated here by markers of systemic inflammation – and eventually affect one’s risk of periodontal inflammation.

Furthermore, our findings are consistent with previous studies emphasizing the role of SEP in linking oral and systemic health outcomes. For example, in a study conducted on middle-aged American army men during the Vietnam War, income and education were shown to be stronger explanatory factors of the relationship between oral health and all-cause mortality compared to health behaviours (188). Indeed, behavioural explanations of oral and systemic diseases have long been debated by social epidemiologists as factors that
lie along the pathway to disease rather than being “causals” (102, 173). Many calls to enhance oral disease prevention policies such as the London Charter on Oral Health Inequalities and the Global Oral Health Inequalities Research Agenda (GOHIRA) have thus focused on the importance of understanding and tackling “upstream” social and economic factors (2, 23).

In this analysis, indicators of SEP were similarly associated with oral and systemic inflammation, where higher income and education reduced the risk of both inflammatory conditions. This similarity supports the notion that oral and systemic diseases share common causals that lie within the social environment, and once again, speaks to the importance of understanding the underlying mechanisms by which the social environment brings about inflammation.

Our study has the advantage of providing evidence on the role of structural and social factors in oral and systemic inflammation by analyzing a large, nationally representative dataset of American adults and by quantifying the impact of socioeconomic and behavioural factors in the oral-systemic health connection. It further provides insight into the notion of biological embodiment by which social conditions penetrate the body and interact with its functions to bring about disease. However, a few limitations were encountered in this analysis. Variables on oral health behaviours such as tooth brushing were not available in NHANES IV and information on the availability of dental insurance was also limited to a few cycles. Thus, it was not possible to include these variables in the analysis. Dental attendance was however used as a proxy to tooth cleaning and dental insurance. This was based on previous findings that showed individuals who had no dental insurance to have reported the least number of dental visits, according to both American
and Canadian data (189)(190). Also, this study cannot support causal inferencing due to the cross-sectional nature of NHANES IV. Longitudinal studies that can better investigate the causal relationship between social and biological factors in disease outcomes are needed in the future.

While this study aimed to develop a better understanding of the social pathways linking oral and systemic health, more studies will be needed that further investigate other pathways, such as the role of psychosocial stress and health-system related factors in the biological processes leading to oral disease.

Unraveling the mechanisms by which oral and systemic inflammatory diseases are linked has become a critically important yet under-investigated area of biomedical research in general, and dental research in specific. This study has examined how these conditions may be related from a novel angle by adding the social dimension to the equation. Clearly, it is imperative to further investigate how such social factors regulate physiological and pathophysiological processes. This should not only develop a better understanding of how disease occurs, but should also aid in developing novel loci for intervention, leading to more effective prevention policies and therapeutic strategies that essentially target the social determinants of health.
Chapter 6

Study III: Stressed-Out Oral Immunity: A Gateway from Social Adversity to Periodontal Disease?

This manuscript will be submitted to Scientific Reports:

Gomaa N, Nicolau B, Glogauer M, Siddiqi A, Tenenbaum H, Fine N, Quiñonez C.
Stressed-out oral immunity: A gateway from social adversity to periodontal disease?

Abstract

**Background and objectives:** The biopsychosocial pathway has been postulated as a route by which social adversity can “get under the skin” to promote disease and health inequalities. Little is known about whether stress-related biological processes can account for any socioeconomic differences in periodontal disease (PD). This study aims to assess the extent of association between socioeconomic position (SEP), PD and pro-inflammatory oral immune parameters, and the contribution of psychosocial stress and stress hormones to these relationships.

**Methods:** In this cross-sectional study, SEP was characterized by annual household income and educational attainment. Participants completed financial and perceived stress questionnaires and underwent full-mouth periodontal examinations to record probing depths, attachment loss and bleeding-on-probing. Stress hormones (cortisol and alpha-amylase) were assessed in hair and saliva samples. Oral inflammatory load was obtained by counting the number of neutrophils in oral rinse samples and neutrophil functionality was analyzed by assessing the expression of a panel of eight Cluster of Differentiation
(CD) markers using multicolour flow cytometry. Crude associations between SEP and periodontal and oral immune parameters were examined. Hierarchical blockwise Poisson regression models were constructed: first, a model adjusted for age and sex; second model adjusted for the psychosocial factors financial stress, perceived stress and cortisol; and third and final model adjusted for frequency of dental attendance.

**Results:** Socioeconomic differences were evident in PD and immune parameters. Compared to middle and higher income groups, individuals earning less than $20,000/year (low-income threshold in Ontario) – had greater levels of PD, higher counts of pro-inflammatory and hyperactive oral neutrophils which are conducive to further periodontal damage. An association was also observed between PD and immune parameters and higher levels of financial stress, perceived stress and elevated cortisol levels. Multivariable regression models showed that financial stress and cortisol significantly attenuated the socioeconomic differences in PD and immune parameters. The contribution of dental attendance to these socioeconomic differences was of less significance.

**Conclusion:** Psychosocial stress may contribute to a pro-inflammatory immune state that is implicated in PD, particularly in individuals exposed to socioeconomic adversity.
6.1 Introduction

The relationship between social adversity and oral health conditions is well established. Despite biomedical advances in the prevention, diagnosis and treatment of oral diseases, the latter continue to place a negative toll on individuals and society (1, 2, 15, 191). Periodontal disease (PD), for example, is one of the most prevalent chronic inflammatory conditions and a main cause of tooth loss in Canada and the world (13, 14, 17). Known for its strong association with major health problems (e.g. cardiovascular disease) (170, 192), PD tends to concentrate in individuals of lower income and education, those who lack dental insurance and those who avoid dental care because of costs (3, 20). Attributed not only to a lack of dental care but also to modifiable social and living conditions, these oral health inequalities have been recognized by several oral health research and policy bodies as unnecessary and unfair, and have as such designated the closure of the social gap in oral health as a priority for action (2, 23).

In order to address oral health inequalities and devise evidence-informed policy solutions, it is critically important to understand the underlying mechanisms that govern disease risk and its inequitable distribution (175). Research in social epidemiology has long focused on socioeconomic indicators, such as income or education, as root or “fundamental” causes of health outcomes, yet has paid less attention to the important intermediary steps therein (35). Several animal and human studies have shown that adverse social exposures influence health through two broad categories of intervening mechanisms: health-relevant behaviours; and psychosocial stress and related cognitive/affective processes (41)(107, 193). Traditionally, health behaviours, such as oral hygiene practices, have been conceived as the “cause” of socioeconomic differences in oral health. However,
the effectiveness of behaviour-targeting interventions in reducing oral health inequalities and the sustainability of their results can be challenged (29, 30). Indeed, such approaches have been postulated to only tackle oral health at the individual micro-level, taking a “reductionist” approach that compartmentalizes the mouth, separating it from the rest of the body. Importantly, behaviour-targeting interventions in general are also suggested to be “victim blaming” in nature, and to possibly widen social gaps in oral health (30, 34).

By contrast, the biopsychosocial model offers a more integrative theoretical orientation, which includes behavioural, psychological and social dimensions in understanding health (41). Initially introduced by Engel to argue against a reductionist biomedical model of disease, it is now one of the dominant views on how social and living circumstances “get under the skin” to influence health outcomes (41). For example, the psychosocial stress generated by an individual’s perception of their position within the socioeconomic hierarchy is linked to the negative health consequences imposed by poor social conditions (43). Physiologically, such stress is associated with higher levels of cortisol release through the consistent activation of the hypothalamic pituitary-adrenal axis (HPA), placing a chronic burden on the body’s neuroendocrine and immune functions, a process known to occur in various negative health states, thus providing evidence on the biopsychosocial linkages between social adversity and inflammatory outcomes (68, 84, 194, 195).

In terms of periodontal health, a number of studies have examined its relationship with psychosocial stress (58, 143, 196). For example, allostatic load, a biological indicator of the “wear and tear” placed on several body systems induced by chronic stress has been shown to explain the socioeconomic and racial inequalities in periodontal health (69, 70).
In another study, financial strain was shown to associate with loss of periodontal attachment and alveolar bone loss – a relationship that was shown to be exacerbated by inadequate coping strategies (58). While these studies highlight the role of psychosocial stress in oral health outcomes, less is known about the biological mechanisms by which social factors become embodied to contribute to the pathobiological process in PD (175).

Importantly, oral neutrophils (OPMs) (cells of the innate immune system in the oral cavity that originate from the peripheral circulation) are known to play a significant role in periodontal pathobiology. Recent studies have shown that these cells are increased in number in PD, resulting in a high oral inflammatory load (OIL) and can be pro-inflammatory and hyperactive in patients with PD (156, 178, 197). In this regard, the stress hormone cortisol, is known to influence circulating neutrophils by inhibiting apoptosis (cell death) and subsequently extending the half-life of these short-lived cells (198). Cortisol also enhances the induction of neutrophil production from hematopoietic stem cells; another mechanism by which it contributes to increased neutrophil counts in the circulation (198, 199). Given this, it is plausible that stress due to adverse socioeconomic exposure may be a contributor to the regulation of OPMN counts and activity in PD. Guided by a hypothetical conceptual model (Figure 6-1), we sought to investigate the association between socioeconomic position (SEP), PD and parameters of oral innate immunity, and the extent to which psychosocial factors mediate these associations.
Figure 6-1. Hypothetical conceptual model
6.2 Methods

6.2.1 Study design, participants and procedures

This cross-sectional study was conducted and reported in accordance with the STROBE guidelines for observational investigations (200). Participants were recruited through advertisements placed in the Faculty of Dentistry, University of Toronto clinics and in three private dental practices within the City of Toronto. Adults aged 20-59 years were eligible for participation if they had no current chronic, autoimmune or inflammatory disease (e.g. diabetes, rheumatoid arthritis); and in the past three months, had not undergone prolonged corticosteroid, antibiotic, probiotic or prescription medication, hair treatment/coloring, periodontal cleanings or surgeries. All study participants were given a two-hour morning appointment at the Faculty of Dentistry, where they were taken through the informed consent process. Information on participants’ socioeconomic and living conditions, health-related behaviours, self-rated general and oral health, access to medical and dental health services were collected, based on questionnaires used for data collection in the Canadian Health Measures Survey (201). Perceived and financial stress scales were also administered. Participants were asked to give hair and saliva samples for the assessment of the stress hormones cortisol and salivary alpha-amylase, respectively. Oral rinse samples were used to obtain oral innate immune parameters, including oral inflammatory load (total OPMN counts) and OPMN immunophenotype and function. A full-mouth periodontal examination was then undertaken. All participants received an honorarium of $50 and individualized oral hygiene instructions. The study was approved by the Health Sciences Research Ethics Board (protocol number 31493) at the University
of Toronto and all procedures were carried out in accordance with the Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans (202).

6.2.2 Exposure variables

6.2.2.1 Socioeconomic position

Annual household income was measured based on the respondents’ self-reported highest household income in the past year before taxes. Accordingly, participants were categorized into three groups: lower income (less than $20,000); middle income ($20-79,000) and higher income (more than $80,000). The cut-off of $20,000/year was selected based on the Ontario limit for low-income, and according to which eligibility for some social assistance programs is determined. Educational attainment was measured according to self-reported highest household education level, based on the number of years of schooling, or less than high school, completed high school, and more than high school (postsecondary/university education). As an indicator of material circumstances within the SEP construct that reflects access to resources, home ownership was categorized based on whether participants owned or rented their home, or resided in a dwelling without payment (e.g. living with parents, living in shelter, etc.).

6.2.2.2 Financial and perceived stress

Financial stress was measured using the Financial Stress Scale, based on an adaptation of the economic strain measure by Pearlin et al. (203), which has also been used in recent studies (204, 205). This eight-item scale assesses the subjective perception of one’s personal finances, money problems, difficulty paying bills, lack of discretionary income, and limited opportunities. Our adaptation of the scale included a question on the financial
barriers to dental care (Cronbach’s alpha 0.81). A binary variable (0=low, 1=high financial stress) was generated based on the scale of 0-17 with a cut-off using a minimum score of ten based on previous research (205). Perceived stress was assessed using the Perceived Stress Scale, which is a widely used, psychometrically validated (Cronbach’s alpha= 0.89), 10-item questionnaire developed by Cohen et al. (206), which measures the degree to which participants consider their lives to be unpredictable, uncontrollable and overloaded (206).

6.2.2.3 Hair cortisol

A hair sample (approximately 100 strands, ≥20 mg of hair) of at least 3 cm in length was collected. The sample was cut using fine scissors, as close as possible to the scalp and from the vertex posterior region (207, 208). Hair samples were attached to a sheet of paper using painter’s tape. The hair root was marked and the collection date and participant identification number were recorded (208). The sample was then enclosed in a Ziploc bag and stored at room temperature, until shipped to the laboratory for analysis. The most proximal 3 cm hair segment was cut, placed into a glass vial, labeled and weighed to ensure a minimal weight for analysis of 3 mg. Hair was then washed twice by immersing the segments in 3 ml of isopropanol, followed by a 3-minute incubation on a shaker at 0.11 g (100 rpm) at room temperature and then analyzed using a commercially available salivary cortisol enzyme immunoassay kit (Alpco Diagnostics, Salem, NH). Hair cortisol (HC) was further dichotomized (normal and high) based on whether HC concentration levels fell above the reference range for normal (17.7-153.2 pg/mg) (209).
6.2.2.4 Salivary alpha-amylase

The enzyme salivary alpha-amylase is a biomarker for stress-related changes in the body that reflect the activity of the sympathetic nervous system. Participants were asked not to eat, drink, brush or floss their teeth at least one hour to their appointment, and unstimulated/passive saliva was collected into 2 ml cryovials (Salimetrics, State College, PA). The samples were stored in -20° C freezer. For analysis, samples were thawed and vortexed, and then centrifuged at 2500 rpm for 15 minutes. An overall 1:200 dilution was achieved in two serial steps: first, 10 μl saliva was added to 90 μl diluent (1:10 dilution), followed by 10 μl of this initial dilution added to 190 μl of diluent (1:20 dilution, making a 1:200 dilution overall). The AgileReader™ ELISA Plate Reader (ACTgene, Inc., Piscataway, NJ) was used for the kinetic salivary alpha-amylase assay. Absorbance was measured at 405 nm at 1 min and then again at 3 min after substrate addition. The difference between these absorbances was calculated and multiplied by 328 to yield enzyme activity, based on the formula provided by the manufacturer which accounts for the light path of the provided wells, millimolar absorptivity of 2-chloro-p-nitrophenol, and dilutions.

6.2.3 Outcome variables

6.2.3.1 Oral inflammatory load

Participants were asked to swish for 30 seconds using 3 ml of isotonic sodium chloride solution 0.9% (Baxter, Toronto, ON, Canada) before any probing of the gingival tissues or manipulation of the oral tissues to avoid initiating gingival bleeding that may interfere with the results. The participants were asked to expectorate the rinse sample into a sterile 50 ml
tube and repeat this procedure 10 times (for a total of 30 ml) with 2.5-minute intervals between each rinse sample. The sample was kept on ice and transferred to the laboratory for processing within 3 hours of collection. In the lab, the sample was fixed and centrifuged at 2500 rpm for 10 minutes. The cell pellet was re-suspended in phosphate buffer saline (PBS) and filtered to get an aliquot of neutrophils. The neutrophil concentration was finally obtained using the Cell and Particle Counter (Beckman Coulter, USA), and reported as n x10⁶/ 30 ml of saliva (where n is the number of OPMNs).

6.2.3.2 Pro-inflammatory OPMN function

The samples were then centrifuged at 1000 rpm, 2500 rpm, and 3000 rpm for 5 minutes each and the cell pellet was re-suspended in FACS buffer and blocked for 20 minutes on ice. Antibodies for a panel of eight CD markers (CD66 APC, CD63 PerCP, CD55 FITC, CD16 AF700, CD11b APC-Cy7, CD18 BV421, CD64 PE, CD14 PE-Cy7) were added. These have been previously identified on oral neutrophils and have been implicated in neutrophil function and activity (178). The samples were incubated in the antibody for 30 minutes in the dark, and then washed with FACS buffer and stored at 4°C until analyzed using the flow cytometer within five days of processing. The results were analyzed using FlowJo Single Cell Analysis software. Participants were then categorized as having a positive pro-inflammatory immunophenotype based on whether they exhibited a high expression of one or more CD markers, as established in previous research (178). The percent of pro-inflammatory OPMNs was also used. The functions of the CD markers selected in this study and the cut-offs used for characterizing pro-inflammatory OPMNs are summarized in the supplemental Table S-6-1.
6.2.3.3 PD parameters

A full-mouth dental examination was performed for each participant using a UNC–15 periodontal probe, by two calibrated dental professionals. Periodontal measures recorded included probing depths (PRD), bleeding on probing (BOP) and clinical attachment loss (AL) at six sites for each tooth. *Kappa* scores for intra and inter-examiner reliability were 0.85 and 0.78, respectively, demonstrating a high degree of examiner reliability and reproducibility. The extent of PD was calculated as the ratio of sites with the periodontal parameter over the total number of periodontal sites probed. The periodontal parameters used in this study were: the extent of ≥4 mm PRD; the extent of ≥3 mm AL; and the extent of BOP. These parameters were operationalized based on measures that have been previously used in population studies (18, 69, 211).

6.2.3.4 Covariates

The covariates accounted for in this study included frequency of tooth brushing, smoking, alcohol consumption, frequency of dental visits.

6.2.4 Statistical analysis

Descriptive statistics were applied and correlations between periodontal and oral innate immune parameters were assessed using Spearman’s rank correlation coefficient. The extent of PD was measured as the number of sites with the periodontal condition to the total number of periodontal sites probed, resulting in a count dependent variable. The nature of PD parameters as dependent variables violated the assumptions for linear regressions, we thus used Poisson regression models, after fulfilling the assumptions for
these models. Logistic regression models were used for the dichotomous variable of pro-inflammatory neutrophil function. Data modeling proceeded in several steps. First, we assessed the crude associations between SEP and periodontal and immune parameters. The association between psychosocial stress factors, such as financial stress, perceived stress and stress hormones (cortisol and alpha-amylase) and periodontal and immune parameters and were also assessed. To assess the contribution of psychosocial stress factors to the crude associations between SEP and periodontal and immune parameters, we then constructed a series of hierarchical block-wise Poisson regression models to assess the incidence rate ratio: (1) a model adjusted for age and sex, (2) a model that added psychosocial factors (financial and perceived stress and cortisol), and (3) finally a third model that adjusted for the frequency of dental attendance. Data were entered into the models as follows:

Model 1: \[ Y = \alpha + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 \]

Model 2: \[ Y = \alpha + (\beta_1X_1 + \beta_2X_2 + \beta_3X_3) + (\beta_4X_4 + \beta_5X_5) \]

Model 3: \[ Y = \alpha + (\beta_1X_1 + \beta_2X_2 + \beta_3X_3) + (\beta_4X_4 + \beta_5X_5) + \beta_6X_6 \]

Where variable \( X_1 = \text{SEP}; X_2 = \text{age}; X_3 = \text{sex}; X_4 = \text{financial stress score}; X_5 = \text{cortisol}; X_6 = \text{frequency of dental attendance} \)

For robustness, we ran the same models using logistic regression with PD operationalized as a dichotomous variable. While oral health behaviours such as smoking, alcohol consumption and the frequency of tooth-brushing are known to impact oral health outcomes and are important factors to control for, our sample lacked variability in these covariates, thus they were eliminated from further analyses. All statistical analyses were conducted using STATA 14.1 (College Station, TX, USA).
6.3 Results

6.3.1 Characteristics of study participants

A total of 95 participants were recruited to this study out of which ten were excluded from analysis due to short (<3 cm) hair samples or missing data. Table 6-1 summarizes the characteristics of the study sample. Participants were mostly middle-aged adults (39.7±12.4 years; m±sd) with a slightly higher percent of females (52%). Most participants (75%) had completed at least post-secondary education. There was an adequate distribution of household income, where almost one third of participants (31%) made less than $20,000 annually, whereas 29.4% had an annual household income of $80,000 or more. Sociodemographic characteristics were generally shown to be comparable to data from the Toronto Census Profile 2016 (212) (Table S-6-2).

Less than half of the sample (48%) reported high financial stress levels, while above threshold cortisol was found in 20% of the sample. The majority of participants (66%) had ≥4 mm periodontal pocketing and ≥3 mm of attachment loss on at least 20% of the total sites probed. However, there was no significant variability in the sample in terms of tooth brushing, smoking or alcohol consumption. Participants with PD showed a two-fold increase in the average oral neutrophil counts (5.0±2.3x10^6 cells/sample) compared to healthy individuals (2.6±1.6x10^6 cells/sample). Spearman’s rank correlation coefficients revealed positive correlations between PD and oral immune parameters (Table S2).
Table 6-1. Characteristics of study sample participants

<table>
<thead>
<tr>
<th>Study participants (n=85)</th>
<th>m± sd; n (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.7±12.4</td>
<td>20-59</td>
</tr>
<tr>
<td>Sex (females)</td>
<td>44 (51.7)</td>
<td>-</td>
</tr>
<tr>
<td>Marital status (married)</td>
<td>43 (50.5)</td>
<td>-</td>
</tr>
<tr>
<td>Annual household income ($)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; $20,000</td>
<td>26 (30.5)</td>
<td></td>
</tr>
<tr>
<td>$20-79,000</td>
<td>34 (40.0)</td>
<td></td>
</tr>
<tr>
<td>≥ $80,000</td>
<td>25 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school (&lt;12 years)</td>
<td>11 (12.9)</td>
<td></td>
</tr>
<tr>
<td>High school (12 years)</td>
<td>10 (11.7)</td>
<td></td>
</tr>
<tr>
<td>Post-secondary/University (&gt;12 years)</td>
<td>64 (75.2)</td>
<td></td>
</tr>
<tr>
<td>Home ownership</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rents home</td>
<td>55 (64.7)</td>
<td></td>
</tr>
<tr>
<td>Owns home with mortgage</td>
<td>7 (8.2)</td>
<td></td>
</tr>
<tr>
<td>Owns home with no mortgage</td>
<td>3 (3.5)</td>
<td></td>
</tr>
<tr>
<td>Occupied without payment</td>
<td>20 (23.5)</td>
<td></td>
</tr>
<tr>
<td>Self-rated general health</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good/very good</td>
<td>57 (67.0)</td>
<td></td>
</tr>
<tr>
<td>Satisfactory</td>
<td>26 (30.5)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>2 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Self-rated oral health</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>13 (15.1)</td>
<td></td>
</tr>
<tr>
<td>Satisfactory</td>
<td>47 (55.2)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>25 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Availability of dental insurance (no)</td>
<td>56 (65.8)</td>
<td></td>
</tr>
<tr>
<td>Avoid dental visits due to cost (yes)</td>
<td>49 (57.6)</td>
<td></td>
</tr>
<tr>
<td>Dental attendance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>4 (4.7)</td>
<td></td>
</tr>
<tr>
<td>Only for emergency</td>
<td>25 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Less than once a year</td>
<td>17 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Once or more than once a year</td>
<td>43 (45.8)</td>
<td></td>
</tr>
<tr>
<td>Tooth brushing (at least twice a day)</td>
<td>59 (69.3)</td>
<td></td>
</tr>
<tr>
<td>Smokers a (currently or previously)</td>
<td>8 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Alcohol (consumption on a regular basis)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Perceived stress score</td>
<td>17.5±5.7</td>
<td>1-31</td>
</tr>
<tr>
<td>Financial stress score</td>
<td>8.8±3.8</td>
<td>0-17</td>
</tr>
<tr>
<td>Hair cortisol (pg/mg)</td>
<td>68.1±55.9</td>
<td>5.4-250.3</td>
</tr>
<tr>
<td>Extent of ≥4 mm pockets (%)</td>
<td>17.7±16.9</td>
<td>0.0-67.9</td>
</tr>
<tr>
<td>Extent of ≥5 mm pockets (%)</td>
<td>2.9±6.5</td>
<td>0.0-30.2</td>
</tr>
<tr>
<td>Extent of ≥3mm attachment loss (%)</td>
<td>19.8±30.0</td>
<td>0.0-100.0</td>
</tr>
<tr>
<td>Extent of bleeding on probing (%)</td>
<td>29.5±18.9</td>
<td>2.9-78.2</td>
</tr>
<tr>
<td>Oral inflammatory load (x 10⁶)</td>
<td>4.1±2.4</td>
<td>0.4-11.0</td>
</tr>
<tr>
<td>CD66 (x 10³) (MFI)</td>
<td>12.3±10.0</td>
<td>1.7-50.9</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>CD64 (x 10³) (MFI)</td>
<td>3.5±5.7</td>
<td>0.3-32.4</td>
</tr>
<tr>
<td>CD63 (x 10³) (MFI)</td>
<td>11.7±8.2</td>
<td>3.2-42.2</td>
</tr>
<tr>
<td>CD55 (x 10³) (MFI)</td>
<td>4.6±4.2</td>
<td>0.4-21.8</td>
</tr>
<tr>
<td>CD18 (x 10³) (MFI)</td>
<td>7.9±4.5</td>
<td>0.5-28.3</td>
</tr>
<tr>
<td>CD16 (x 10³) (MFI)</td>
<td>2.8±2.0</td>
<td>0.2-13.5</td>
</tr>
<tr>
<td>CD14 (x 10³) (MFI)</td>
<td>1.6±1.4</td>
<td>1.1-7.9</td>
</tr>
<tr>
<td>CD11b (x 10³) (MFI)</td>
<td>2.7±2.1</td>
<td>3.7-10.9</td>
</tr>
<tr>
<td>Pro-inflammatory OPMNs (%)</td>
<td>71.3±17.9</td>
<td>32.6-98.2</td>
</tr>
<tr>
<td>Pro-inflammatory OPMN immunophenotype a</td>
<td>63 (74.1)</td>
<td>-</td>
</tr>
</tbody>
</table>

MFI: geometric mean fluorescence intensity
*aExhibiting high expression of one or more CD marker
6.3.2 Higher SEP protects against PD and the risk of a pro-inflammatory oral immunity

Compared to those earning less than $20,000 per annum, individuals in the middle and higher SEP groups had a significantly lower probability for periodontal pocketing, bleeding on probing and oral inflammatory load (Figure 6-2). Also, individuals of the more affluent groups were significantly less likely to have a pro-inflammatory immunophenotype (OR= 0.1 95%CI 0.0, 0.7) (Figure 6-3). A similar pattern of socioeconomic differences was observed in periodontal and immune parameters by educational attainment, where participants who completed at least 12 years of education were less likely to have a pro-inflammatory OPMN immunophenotype and a lower probability of a high oral inflammatory load, compared to their counterparts of higher educational attainment. Home ownership on the other hand, showed no significant association with these outcomes.
Figure 6-2. Socioeconomic differences in the probability of periodontal pocketing (PRD), bleeding on probing (BOP) and oral inflammatory load (OIL). Ref: Reference group.
Figure 6-3. Representative histograms of geometric mean fluorescence intensities showing the differences in pro-inflammatory OPMNs between lower SEP (grey) and higher SEP (blue).
6.3.3 PD and risk of proinflammatory immunity increase with financial stress, perceived stress and cortisol

Participants with higher financial stress exhibited a two-fold greater probability of periodontal pocketing (IRR=2.2, 95% CI 1.9, 2.5), and almost 1.5 times greater probability of bleeding on probing (95% CI 1.4, 2.7) and oral inflammatory load (Table 6-2). Similarly, higher cortisol was associated with an increase in oral inflammatory load (IRR= 1.4, 95% CI 1.1, 2.8) and a five-fold increase in the likelihood of having a proinflammatory immunophenotype (95% CI 1.4-10.9). Perceived stress was also significantly associated with PD, but not with immune parameters (Table 6-2).

6.3.4 Effects of financial stress, perceived stress and cortisol on the socioeconomic differences in PD and oral immunity

Adjusting for financial stress, perceived stress and cortisol partly explained the socioeconomic differences in periodontal pocketing, bleeding-on probing and oral inflammatory load by an estimate of 20% between lower and middle income categories (Model 1: IRR=0.5 95% CI 0.3, 0.5; Model 2: IRR= 0.6, 95% CI 0.5, 0.7) and lower and higher income ones (Model 1: IRR=0.4 95% CI 0.3, 0.6; Model 2: IRR=0.6 95% CI 0.5, 0.7). Clinically, this difference accounts for approximately 5.6 teeth with PD. Financial stress and cortisol were also shown to significantly attenuate the likelihood of proinflammatory immune function between lower and middle SEP (Model 1: OR= 0.1 95% CI 0.0, 0.7; Model 2: 0.2 95% CI 0.0, 1.8) and lower and higher SEP (Model 1: OR= 0.1 95% CI 0.0, 0.9; Model 2: 0.3 95% CI 0.04, 2.3). Further adjusting for dental attendance in Model 3 resulted in an attenuation of incidence rate ratios by
less than 7% (<2 teeth), making its contribution to the socioeconomic differences in PD observed in this sample less significant (Figure 6-4). Similarly, the effect of dental attendance on the SEP differences in oral inflammatory load and the likelihood for a pro-inflammatory oral immune function was less significant (Table 6-3). Meanwhile, financial stress and cortisol were of less significance in explaining the socioeconomic differences observed by educational attainment.
### Table 6-2. Financial and perceived stress and cortisol associate with PD and oral immune parameters

<table>
<thead>
<tr>
<th>Periodontal disease; immune parameters</th>
<th>FSS IRR (95% CI)</th>
<th>PSS IRR (95% CI)</th>
<th>HC IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRD</td>
<td>2.2*** (1.9, 2.5)</td>
<td>1.0 $^&lt;$ (1.0, 2.3)</td>
<td>1.2** (1.1, 2.4)</td>
</tr>
<tr>
<td>BOP</td>
<td>1.6*** (1.4, 2.7)</td>
<td>1.9* (1.1, 2.1)</td>
<td>1.1* (1.0, 2.2)</td>
</tr>
<tr>
<td>OIL</td>
<td>1.7*** (1.3, 2.2)</td>
<td>0.9 $^&lt;$ (0.7, 2.1)</td>
<td>1.4** (1.1, 2.8)</td>
</tr>
<tr>
<td>PROINFL $^a$</td>
<td>4.6* (1.3, 16.1)</td>
<td>2.3 $^&lt;$ (0.7, 7.2)</td>
<td>5.5* (1.4, 10.9)</td>
</tr>
</tbody>
</table>

PRD: extent of ≥ 4mm probing depth; BOP: bleeding on probing; OIL: oral inflammatory load; PROINFL: pro-inflammatory immunophenotype; FSS: financial stress; PSS: perceived stress; HC: hair cortisol. $^a$Reported as odds ratios (95% confidence interval). ***p<0.001, **p<0.01, *p<0.05, $^<$ non-significant
**Figure 6-4.** The effect of financial stress, perceived stress and cortisol on socioeconomic differences in (A) the extent of ≥ 4mm periodontal pockets and (B) oral inflammatory load; Model 1: adjusted for age and sex; Model 2: adjusted for financial stress and cortisol; Model 3: additionally adjusted for dental attendance. Note the greater change in incidence rate ratios between Models 1 and 2 for both middle and higher SEP categories, compared to the difference in Model 3. Ref: Reference group.
Table 6-3. The effect of psychosocial stress on the socioeconomic differences in periodontal disease and pro-inflammatory phenotypes

<table>
<thead>
<tr>
<th>Periodontal disease; immune parameters</th>
<th>Model 1 IRR (95% CI)</th>
<th>Model 2 IRR (95% CI)</th>
<th>Model 3 IRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Middle</td>
<td>Higher</td>
</tr>
<tr>
<td>PRD</td>
<td>Ref</td>
<td>0.5 ***</td>
<td>(0.3, 0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4 ***</td>
<td>(0.3, 0.6)</td>
</tr>
<tr>
<td>BOP</td>
<td>Ref</td>
<td>0.6 **</td>
<td>(0.5, 0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 ***</td>
<td>(0.4, 0.6)</td>
</tr>
<tr>
<td>OIL</td>
<td>Ref</td>
<td>0.6 ***</td>
<td>(0.47, 0.76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ***</td>
<td>(0.36, 0.65)</td>
</tr>
<tr>
<td>PROINFL a</td>
<td>Ref</td>
<td>0.1*</td>
<td>(0.0, 0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1*</td>
<td>(0.0, 0.9)</td>
</tr>
</tbody>
</table>


*Reported as odds ratios (95% confidence interval).

***p<0.001, **p<0.01, *p<0.05, ¶ non-significant
6.4 Discussion

Biologically-grounded explanations of the social determinants of health are important for informing health policy makers as well as other consumers of health research. (35) Stemming from a much needed paradigm shift towards interventions that target the “fundamental causes” of disease, the process of biological embodiment through which social factors can influence behaviour and biological function has gained increasing attention (36).

Utilizing a biopsychosocial approach, our study is the first to investigate how structural factors such as SEP “get under the skin”, translating into pathobiological processes that favour the development of PD. Our work extends existing knowledge on the impact of the social environment on oral health in several ways. First, we examine both psychometrically and physiological measures of stress in relation to oral health parameters. Second, where most previous studies have focused on clinical signs of PD, we examine the effect of stress on measures of the innate immune system – a pivotal player in PD. Third, we incorporate the study of immune biomarkers in understanding the biopsychosocial mechanisms that underlie socioeconomic differences in oral health outcomes. In doing so, our study has important key findings. Conforming with previous work on social inequalities in oral health in various populations, (18, 213) socioeconomic differences in PD were present in this sample. In addition, it was demonstrated that these differences extend to the immune system, where individuals of lower SEP are more likely to have pro-inflammatory immune cells that can make them more vulnerable to more periodontal tissue destruction than individuals with average or higher SEP (178). These findings are consistent with previous work in this area showing that social exposures such as low SEP and social isolation relate to immune system
changes that can generally make individuals more prone to disease (92-94, 214). These immune system changes, however, varied from impaired to hyperactive to an aging immune system (as detected by short leukocyte telomere lengths) (215, 216). This suggests that there are different mechanisms by which adverse social exposures can become embodied, and which can possibly be influenced by the nature of the social exposure. These include but are likely not limited to various host factors such as immune system vulnerability, stress coping mechanisms, health behaviours and the resultant health condition.

Interestingly, in this sample, the likelihood of participants having pro-inflammatory hyperactive immune cells associated positively with higher financial stress and levels of cortisol. From the stance of biological plausibility, cortisol is known to regulate leukocyte subsets and to positively correlate with neutrophil numbers in the circulation (217). Increased cortisol doses have been shown to impair apoptosis and to increase the half-life of circulating neutrophils in experimental studies. While there is scarce evidence on the effect of cortisol on OPMNs, it may be possible that a similar mechanism would explain the positive association observed in our sample between oral inflammatory load and cortisol levels. Meanwhile, the effect of cortisol on neutrophil function has been debated in the literature. Cortisol is known to reduce cell-cell adhesion and impair circulating neutrophil function (e.g. superoxide generation) thereby permitting infection to occur more readily. However, in inflammatory diseases, it has been suggested that there are stress-related changes in the sensitivity of immune cells to regulation by cortisol. For example, disruption of leukocyte distributional sensitivity to cortisol and diminished glucocorticoid receptor-mediated gene expression have been linked to chronic stress and HPA baseline activity, in both animal and human models. In line with this, our findings suggest that cortisol has a positive relationship with OPMN
degranulation and adhesion properties (as evidenced by the elevated expression of CD66a, CD63 and CD11b and hence the pro-inflammatory immunophenotype). This may be attributed to differences in functionality and transcriptome between neutrophils in the oral cavity and those in the circulation, as shown in previous research (197), and might have implications as to how these different groups of cells react to endogenous cortisol. Additionally, it may be plausible that OPMNs have developed glucocorticoid resistance in a mechanism similar to that observed in previous studies on circulating neutrophils with prolonged exposure to cortisol due to stress (92, 214). Further studies in this area will be needed to assess the impact of cortisol on OPMNs.

Importantly, our results show that financial stress and cortisol explain socioeconomic differences in PD and oral immune parameters. In his landmark work “Sick individuals and sick populations”, Geoffrey Rose pointed out that small shifts in the distribution of risk could make large differences in the health status of a population (218). In this way, our findings suggest that shifting individuals towards more socioeconomic stability can potentially and significantly reduce the socioeconomic gap and therefore lead to reduced risks for periodontitis by reducing the risk for financial stress and related biological factors, i.e. stress hormones. Yet, as observed in this sample, as one goes up the socioeconomic hierarchy, the role of other factors such as behaviours (e.g. attending for dental treatment) can become evident, further illustrating some of the socioeconomic differences between lower and higher income groups.

Unlike the case for the income gradient in periodontal disease referred to above, the contribution of psychosocial stress indicators was of less significance in explaining socioeconomic differences in periodontal disease by educational attainment, suggesting that
stress has a more important role in the periodontal disease process in individuals with lower income compared to those with lower education. This observation may likely be due to the notion of status incongruity which is the general mismatch between income and education in the population, and further points out that there are possibly non-stress-related factors that contribute to the inequalities in periodontal health by levels of education.

Our sample was homogenous in terms of oral health behaviours, where all participants self-reported as following oral hygiene practices when asked about tooth-brushing habits and whether they were current or past smokers. Despite these oral health behaviours not being a significant factor in this sample, there were evident socioeconomic differences in PD and oral immune parameters. This indicates that such inequalities are not only due to behavioural differences between individuals, and is in accordance with previous work that showed oral health behaviours as having a limited contribution to oral health inequalities (160).

An important aspect of this study concerns the similarities in the social and psychosocial mechanisms between PD and previously studied inflammatory diseases. Biologically, PD is considered an accessible surrogate for studying the pathobiology of inflammation, due to its mimicry of several inflammatory conditions (27, 219). However, from a social standpoint, PD may be perceived differently when compared to other inflammatory conditions, likely because of its strong link with poor social stance and individually-based behaviours (i.e. poor oral hygiene practices) (102, 220). Furthermore, given that PD is often socially perceived as a disease of poverty and social deprivation, individuals with poor periodontal health may also experience social exclusion, discrimination and stigma (33, 220). One could argue that such perceived social differences (despite the
biological similarities between periodontal and other inflammatory conditions) have, in a way, contributed to the separation of the mouth from the body, which has implications for policy and practice (221). Nevertheless, our study shows that PD, as other inflammatory diseases, is socially stratified and psychosocially determined, thus implicating the need for integrated policy solutions that consider oral and non-oral conditions alike.

Given the exploratory nature of the study, some limitations are present. The cross-sectional design does not allow for causal inference. Additionally, although the sample has a similar sociodemographic profile as the larger population, the modest sample size and the lack of variability in some oral health related behavioural factors, restricts the generalizability of our findings. Future research studies should thus be conducted on larger population-based samples.

Ultimately, this work contributes to a better understanding of the biopsychosocial pathways that explain PD, related immune processes, and their socioeconomic patterning. It reveals that the effect of psychosocial stress can vary according to one’s SEP and tends to be more significant in the less affluent. It importantly suggests that social policies that alleviate socioeconomic and psychosocial stressors may contribute to reducing the burden of oral disease and related oral health inequalities.

6.5 Acknowledgments

We would like to acknowledge the support of the funding agencies that made this work possible: NG is a recipient of the Ontario Graduate Scholarship (OGS) and the Queen Elizabeth II Graduate Scholarship in Science and Technology (QEII GSST) from the Government of Ontario. AS is a Canada Research Chair in Population Health Equity. BN is a
Canada Research Chair in Life Course Oral Epidemiology. We are thankful to Ms. Morvarid Oveisi from the Matrix Dynamics Group, University of Toronto for assisting with the flow cytometry analysis.
Table S-6-1. CD marker expression levels to determine oral neutrophil pro-inflammatory immunophenotype

<table>
<thead>
<tr>
<th>CD marker</th>
<th>Proein: function</th>
<th>Cut-off for pro-inflammation (MFI) x10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD66a</td>
<td><em>Ceacam</em>: adhesion, degranulation</td>
<td>14.2</td>
</tr>
<tr>
<td>CD64</td>
<td><em>Fc-γRI</em>: high-affinity Fc receptor</td>
<td>1.1</td>
</tr>
<tr>
<td>CD63</td>
<td><em>Granulophysin</em>: degranulation</td>
<td>19.6</td>
</tr>
<tr>
<td>CD55</td>
<td><em>DAF</em>: complement inhibitor</td>
<td>6.7</td>
</tr>
<tr>
<td>CD18</td>
<td><em>β2-integrin</em>: adhesion, complement receptor</td>
<td>13.0</td>
</tr>
<tr>
<td>CD16</td>
<td><em>Fc-γRIII</em>: low-affinity Fc receptor</td>
<td>3.8</td>
</tr>
<tr>
<td>CD11b</td>
<td><em>αM-integrin</em>: adhesion, complement receptor</td>
<td>6.7</td>
</tr>
</tbody>
</table>

CD: Cluster of Differentiation  
MFI: mean fluorescence intensity
Table S-6-2. Sociodemographic characteristics of the study sample compared to data from Toronto Census, 2016

<table>
<thead>
<tr>
<th></th>
<th>Study sample</th>
<th>Toronto Census Data, 2016 a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>39.7</td>
<td>40.6</td>
</tr>
<tr>
<td>Females</td>
<td>51.7</td>
<td>40.8</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$20K</td>
<td>30.5</td>
<td>34.8</td>
</tr>
<tr>
<td>$20-79K</td>
<td>40.0</td>
<td>45.7</td>
</tr>
<tr>
<td>≥$80K</td>
<td>29.4</td>
<td>23.8</td>
</tr>
</tbody>
</table>

a Income reported as the total income for the population aged 15 years and over in private households, after taxes.
Table S-6-3. Spearman’s rank correlations between periodontal and oral immune parameters

<table>
<thead>
<tr>
<th>PD</th>
<th>Oral innate immune parameters (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral inflammatory load</td>
</tr>
<tr>
<td>≥ 4mm PD (%)</td>
<td>0.7</td>
</tr>
<tr>
<td>≥ 3mm AL (%)</td>
<td>0.4</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

PRD: periodontal pocketing; AL: clinical attachment loss; BOP: bleeding on probing

r: Spearman’s rank correlation coefficient
Chapter 7

General Discussion

The overarching aim of this dissertation was to develop an understanding of the pathobiological pathways linking social conditions with oral disease outcomes, and how these can potentially contribute to social inequalities in oral health. The projects included in this dissertation have stemmed from the existing literature and conceptualizations on biological embodiment, extending these concepts to the study of oral health conditions, using different study designs and methods. In doing so, this dissertation has important key findings, as will be outlined in the next section.

7.1 Social-biological interactions are dynamic and reciprocal

Through this dissertation, we have outlined the various theories and the empirical evidence on the links between social and biological factors in oral disease, showing that oral health outcomes may not be merely a pathobiological result of the differences in oral health behaviours, but instead a process by which the dynamicity and reciprocity between social, psychosocial and biological factors interplay, within a biopsychosocial context.

7.2 SEP differences in PD extend to the immune system

This work has importantly demonstrated that the well-known socioeconomic gap in clinical oral disease parameters also extends to the oral and systemic immune systems. More specifically, it provided evidence that individuals exposed to adverse socioeconomic conditions not only exhibit greater levels of the clinical signs of oral disease, such as
periodontal pocketing and bleeding, but are also at a higher risk for a pro-inflammatory immune system that can promote systemic inflammation (as demonstrated by the cumulative inflammatory load and systemic biomarkers in Chapter 5), and that can contribute to further periodontal tissue damage (as demonstrated by OPMN count and functionality in Chapter 6). These findings conform to the general susceptibility view of disease causation, where adverse social exposures can make individuals more vulnerable to disease compared to their more affluent counterparts.

7.3 Psychosocial stress plays an important role in the socioeconomic differences in PD and immune parameters

This work demonstrated that socioeconomic differences in PD and the risk for a pro-inflammatory immune system are explained by high levels of perceived and financial stress, including the inability to cope with life stressors, difficulty in paying bills, and limits to accessing dental care due to costs. This suggests that a biopsychosocial pathway mediates the relationship between social exposures and oral health conditions. This is in accordance with earlier studies in which financial stress was shown to aggravate periodontal pocketing and alveolar bone loss – an effect that was alleviated by stress coping skills (58).

7.4 Activation of the stress pathway is implicated in explaining PD and oral health inequalities

In addition to assessing stress psychometrically, this work is the first to assess the relationship between cortisol and oral innate immunity, and is the first to do so using non-
invasive hair and oral rinse samples. Considering previous work on how cortisol influences peripheral neutrophils, the association observed here between high cortisol levels and the count and functionality of OPMNs indicates that these cells may have potentially undergone a mechanism of glucocorticoid resistance. While further studies on the mechanisms linking glucocorticoid exposure to OPMNs will be needed to better understand this process, the work presented here provides a guide to future studies that aim to understand the stress pathways in oral immune processes.

7.5 A less significant role for oral health behaviours

This work takes into consideration other important factors that are known to contribute to socioeconomic differences in oral health outcomes, such as the frequency of visiting a dentist (dental attendance) – commonly used a proxy for oral health behaviours. In Chapters 5 and 6, the role of dental attendance contributed to the relationship between PD and systemic inflammation compared to structural factors (e.g. poverty to income ratio and educational attainment) but was of less significance in explaining the socioeconomic differences in PD and oral immunity compared to psychosocial factors (e.g. financial stress, perceived stress and cortisol). These observations indicate that the susceptibility to inflammation and related oral health inequalities are possibly not only due to behavioural differences between individuals, but that a larger role can be attributed to social and psychosocial factors. This agrees with previous work that showed oral health behaviours to have a limited contribution to social inequalities in oral health, and conforms with the notion that behaviours are not the “fundamental causes” but are rather mediators that facilitate the impact of poor social and living conditions on oral health.
Collectively, the findings from this dissertation support the biopsychosocial model and its application to understanding the biology of social adversity in oral disease. It suggests that social and psychosocial factors play an important role in the pathobiology of oral diseases, and can extend to the oral and systemic immune system to potentially impact its functioning and ability to fend off disease.

7.6 Public health policy implications

Kubzansky et al, have stated that identifying the biological mechanisms that link social exposures to health outcomes are of critical importance in guiding interventions, establishing pathways and motivating action; adding that the observed associations between social adversity and poor health are often more powerful when accompanied by evidence of the pathobiological pathways mediating these links (47). Indeed, people continue to live, work and function within a social structure that shapes their behaviours and health outcomes (175). The knowledge generated by this work is of particular relevance to oral health policy makers and other consumers of health research seeking to devise effective oral health policies and strategies that can alleviate the individual and the societal burden of oral disease. It demonstrates that psychosocial factors play an important role in the social patterning observed in oral disease and provides biologically-grounded explanations of why oral disease tends to occur disproportionately in the socioeconomically disadvantaged, thus furbishing ground for evidence-informed oral health policies on the “upstream” causes of oral diseases.
7.7 Study limitations

Along the course of this work, a few limitations were encountered, some of which have been discussed within the relevant chapters. First, the projects included in this dissertation have used cross-sectional data. Therefore, there are limits to the interpretation of whether the observed PD and immune outcomes are “stress-induced” and whether social adversity is the “causal” or the “driving” factor. Future studies should thus be directed towards a longitudinal study design, to assess how social exposures over the life course could affect oral health and immune outcomes later in life. Secondly, the lack of oral health behaviour measures in Chapter 5 and the lack of variability in oral health behaviour measures in Chapter 6 has limited our ability to study their role in the oral and systemic immune parameters. However, studying the contribution of dental attendance – a commonly used proxy for oral health behaviours – has partly compensated for this. Finally, the modest sample size in Chapter 6 limits the generalizability of the results and the extrapolation of our findings to the general population. With that said, comparing the sociodemographic characteristics of our sample to recent Toronto census data shows that the sample is representative of the general population on a sociodemographic basis.

Despite these limitations, this dissertation does provide considerable evidence on social-biological relationships that can shape oral health and are in line with and build on previous findings in the literature about the biological embodiment of social conditions in oral disease.
7.8 Future directions

The studies presented in this dissertation have extended the traditional paradigm of biomedical approaches to understanding oral disease, by integrating the role of social factors into the equation, thus building bridges between the social and biological realms in oral health research and creating an opportunity to propel a new line of investigation. Surely, the combination of theoretical and technical advances in social epidemiology and biomedical sciences can act as catalysts to further our understanding of biological embodiment in oral disease and related systemic conditions. As such, some suggestions for future directions that can build on the findings of the current work are outlined below.

7.8.1 Expanding on the “omics” to understand social-biological interactions in oral disease

A mechanism by which social adversity has been suggested to influence biological processes is via the regulation of gene expression through modifications to the epigenome (222). Alterations to DNA methylation patterns can up- or down-regulate gene expression, thus playing a critical role in the disease process. Such epigenetic changes have been attributed to social and environmental exposures that induce stress in both animal and human models (223, 224). Studies to date have examined how adverse social exposures may cause epigenetic changes in the biological stress response (e.g. epigenetic changes responsible for glucocorticoid receptor resistance), with most studies evaluating epigenetic changes in leukocytes (225, 226). Interestingly, recent work has also pointed towards the role epigenetics can play in PD pathogenesis (227, 228), possibly implicating the a
pathway by which epigenetic stress responses may be responsible for PD susceptibility. Given the findings of this dissertation (Chapter 6) on the relationship between cortisol and OPMN function, it is plausible there are underlying epigenetic mechanisms at play.

Additionally, stress is known to be centrally regulated, and has been shown to associate with hippocampal and amygdalar structural changes, thereby adversely affecting neurocognitive processes (60, 67, 120, 229). Poor social conditions and adverse early life experiences have been shown to interact with alleles of genes to affect epigenetic changes in the brain and other body systems (230). Collectively, the advances in the realm of “omics” research and the progress in the capacity to measure the effect of stress on a variety of biological systems and processes, including brain structure and function, research on such mechanisms has become a promising area of study to further our understanding of social-biological interactions in oral and related systemic health conditions.

7.8.2 Supporting causal inferences

Most current research in the area of biological embodiment is based on observational, cross-sectional studies. The work presented in this dissertation can act as a cornerstone for prospective work that aims to establish causality between the social and biological factors in oral disease. As such, future studies should be directed towards large population-based samples that use more comprehensive statistical methods that allow for causal inferencing and directionality (e.g. structural equation modeling; directed acyclic graphs). Also, an important strategy is to build conclusions on research that utilizes a range of study designs (e.g. laboratory experimental studies to complement population-level observational
studies). Importantly, longitudinal study designs that take a life course approach are necessary to develop an understanding of the impact of toxic social exposures on biology, and whether there are specific times along the life course in which vulnerability may be amplified (e.g. early childhood exposures).

7.9 Conclusion

To conclude, the work presented in this dissertation provides novel evidence on the concept of biological embodiment in oral health conditions, supporting the biopsychosocial model to oral health and demonstrating that adverse social exposures can “get under the skin” to bring about pathobiological changes in relation to oral and systemic immunity. It has clearly shown that social factors such as SEP can significantly contribute to PD and systemic inflammatory outcomes. It has also demonstrated that psychosocial factors play an important role in the pathobiological processes related to PD through the stress pathway; conforming with the notion that there is a biological plausibility to the link between social adversity and poor oral health. Importantly, this work has emphasized the dynamicity and reciprocity between social and biological factors in oral health outcomes. Surely, understanding the biology of social adversity in oral disease will have significant implications to basic science research that aims to understand the “upstream” causes of such disease, and to policy approaches that can ultimately improve oral health and close the social gaps therein.
References


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158. Maes M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011;35(3):664-75.


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Appendices
Appendix I: Advertisement for participant recruitment

Research Study

Oral Health and Stress
Participants needed for a study on the effect of stress on tooth decay and dental pocketing

- Participants should be 18-59 years old
- Participants will receive a dental examination and individualized oral hygiene instructions
- In-cash compensation for your time will be provided

To find out if you are eligible and to learn more about the study, please e-mail:
oralhealthstress@dentistry.utoronto.ca
Appendix II: Consent form

PART I: Information Letter

Introduction

You are being asked to take part in a research study at the University of Toronto, Faculty of Dentistry. Please read this explanation about the study and its risks and benefits before you decide if you would like to take part. You should take as much time as you need to make your decision. You can always ask the study doctor to explain anything that you do not understand and make sure that all your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish. If you have any questions that come up at later, you can contact the study doctor via the email address or telephone number provided under the contact section of this form. Participation in this study is completely voluntary.

Background and Purpose

Oral disease is one of the most prevalent chronic diseases in Canada, with its burden concentrating largely in Canadian families of lower income and education, who typically lack dental insurance and avoid dental visits because of costs. This is unfair and unnecessary since it is not due to genetic or inherent causes, but to social and living conditions. Previous studies have shown oral disease such as gum disease and dental decay, to be linked to stress, which affects the body’s different systems including hormonal balance and the ability of the body to fend off disease.
Our study aims to understand how social and general life conditions affect the body’s defense mechanisms leading to oral disease. We will examine the relationship between social factors such as income, education and stressful life events and the body’s defenses through counting neutrophils (a type of white blood cell) and determining their type and function in an oral water rinse and blood samples. In addition, we will also study if the latter relate to stress hormone levels that accumulate in the hair. The results of our study will help in explaining the causes of oral disease and will aid in guiding policies towards enhancing the oral health of Canadians and reducing the health gap between the rich and the poor.

**Participant selection**

We are inviting adult males and females (18 to 65 years old) to participate in this study. Individuals will be ineligible to participate if they have a medical systemic condition such as which can interfere with sample collection and the study information. These conditions are:

- Autoimmune disorders (e.g. pemphigus, rheumatoid arthritis)
- Immune-deficiency conditions (e.g. HIV, AIDS)
- Neutrophil pathology (e.g. neutropenia)
- Special needs patients
- Patients that have undergone organ transplants in the past 6 months

**Procedures**
If you agree to participate in the study:

1- You will be asked to complete a medical and dental history questionnaire related to your health with your study doctor, which is designed to take no longer than 5-7 minutes.

2- Next, you will be asked to rinse your mouth once with 10 mL of sterile water for 15 seconds, which will then be collected. Prior to the rinse, you will be instructed not to eat or drink for a minimum of 30 minutes to avoid clearance of the cells (neutrophils) that we are planning to count. You will then be asked to chew on a thin piece of parafilm to stimulate your saliva secretion, and then asked to spit 5 ml of oral fluid in a tube.

3- You will be asked to provide approximately 100 hairs from the back of the head, in a painless, safe and non-invasive procedure, using sterile scissors. No hair will be pulled out.

4- You will then have a complete dental examination similar to a regular dental examination at your dentist.

5- Finally, you will be asked to answer a set of questions about your social and living conditions and your daily life stressors.

6- The findings of your dental examination will be discussed with you and if any oral problems are identified, you will be advised of options regarding follow-up and treatment. In addition, you will receive individualized instructions in oral hygiene (i.e. proper technique of brushing and flossing).

7- The entire procedure should take between 1.5-2 hours, and there are no additional visits or investigations that are required as part of this study.
Risks

There is no foreseeable harm or injury as a result of participating in this study.

Benefits

A comprehensive oral examination has the potential benefit of detecting any oral disease, which may be managed thereafter at a dental clinic. Should any oral problems be identified during the study, you will be notified of your options regarding follow up and treatment. You will also be given individualized oral hygiene instructions to improve your oral health as required.

Confidentiality

If you agree to take part in this study, the information that will be collected for this project will be kept under strict confidentiality. Information about you will be anonymized and will be locked and secured onto a protected desktop on a secure server by the study doctor for 7 years. This information may be used in further studies of similar research purposes to build up on the findings from this study. Any information about you will have a number on it instead of your name. Your information will not be shared with third parties.

Voluntary Participation

Your participation in this research is voluntary. You may decide not to be in this study. Whether you choose to participate or not, if you already are a Faculty of Dentistry patient, all the services you receive at this clinic will continue and nothing will change. You will still be offered the treatment that is routinely offered in this clinic/hospital, which will be
discussed later with your dentist/student. You have the freedom to withdraw even if you have agreed earlier.

**Duration**

You are required to come for a single appointment, two-hours in length, in which personal information, sample collection and oral examination will take place.

**Compensation**

You will be offered an amount of $50 in cash in appreciation for your participation.

**Expenses Associated with Participating in the Study**

You will not have to pay for any of the procedures involved in this study.

**Sharing the Results**

You can always welcome to contact the study doctor in 12-18 months from the date of your appointment, via telephone to email, to learn more about the study results.

**Conflicts of Interest**

There are no conflicts of interest to declare in this study.

**Questions About the Study?**

If you have any questions or concerns now or later, or would like to speak to the study team for any reason, please call the study doctor at (416) 979-4908 (ext. 4490) or email oralhealthstress@mail.utoronto.ca.
If you have any questions about your rights as a research participant or have concerns about this study, you can contact the Office of Research Ethics at ethics.review@utoronto.ca or call 416-946-3273.
PART II: Certificate of Consent

I have read the foregoing information. I have had the opportunity to ask questions about it, and questions that I have asked have been answered to my satisfaction. I understand that my anonymized information will be retained and may be used for future studies. I consent voluntarily to take part as a participant in this study.

__________________________________________

Print Name of Participant

__________________________________________

Signature of Participant Date (DD/MM/YYYY)
Statement by the researcher/study doctor

I have accurately read out the information sheet to the potential participant, and made sure that the participant understands the procedures, potential risks and benefits.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

__________________________

Print Name of Researcher

__________________________

Signature of Researcher Date (DD/MM/YYYY)
Appendix III. Sociodemographic questions

Now, I will ask you some questions about yourself and your health, it shouldn’t take longer than 15 minutes, ok?

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you married or live with a partner?</td>
<td>a. Yes</td>
</tr>
<tr>
<td></td>
<td>b. No</td>
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<tr>
<td>How many children under the age of 16 are in your family?</td>
<td></td>
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<tr>
<td>How many people live in your household?</td>
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<td>What is the highest level of schooling you have completed?</td>
<td>1: Primary (0-6 years)</td>
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<td></td>
<td>2: High school without graduation</td>
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<td></td>
<td>3: High with graduation</td>
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<td></td>
<td>4: Community college/ technical school</td>
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<td></td>
<td>5: University degree/bachelors or equivalent</td>
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<td></td>
<td>6: Graduate degree</td>
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<tr>
<td>Thinking about the person in your household with the highest level of schooling, what is the highest level of schooling they have completed?</td>
<td>1: Primary (0-6 years)</td>
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<tr>
<td></td>
<td>2: High school without graduation</td>
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<tr>
<td></td>
<td>3: High with graduation</td>
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<td></td>
<td>4: Community college/ technical school</td>
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<td></td>
<td>5: University degree/bachelors or equivalent</td>
</tr>
<tr>
<td></td>
<td>6: Graduate degree</td>
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<tr>
<td>How often do you usually see a dental professional, such as a dentist, a dental hygienist?</td>
<td>1: More than once a year (for check-ups or treatment)</td>
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<tr>
<td></td>
<td>2: About once a year (for check-ups or treatment)</td>
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<td></td>
<td>3: Less than once a year</td>
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<td>4: Only for emergency care</td>
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<td>5: Never</td>
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<td>8</td>
<td>RF</td>
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<td>9</td>
<td>DK</td>
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</table>

When was the last time you saw a dental professional, such as dentist or a dental hygienist?

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<tbody>
<tr>
<td>1</td>
<td>Less than 1 year to 1 year ago</td>
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<td>2</td>
<td>More than 1 year to 2 years ago</td>
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<tr>
<td>3</td>
<td>More than 2 years to 3 years ago</td>
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<td>4</td>
<td>More than 3 years to 4 years ago</td>
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<td>5</td>
<td>More than 4 years to 5 years ago</td>
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<td>6</td>
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<td>7</td>
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How often do you brush your teeth?

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<tr>
<td>1</td>
<td>Twice a day or more</td>
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<td>2</td>
<td>Once a day</td>
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<td>3</td>
<td>Less than once a day</td>
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<td>5</td>
<td>Never</td>
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<td>8</td>
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At the present time, do you smoke cigarettes frequently, occasionally or not at all?

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<tr>
<td>1</td>
<td>Daily</td>
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<td>2</td>
<td>Occasionally</td>
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<td>3</td>
<td>Not at all</td>
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<td>8</td>
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In the past 12 months:

Have you had any pain in your teeth or mouth?

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<td>1</td>
<td>Never</td>
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<tr>
<td>2</td>
<td>Hardly ever</td>
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<td>3</td>
<td>Occasionally</td>
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<td>Rating</td>
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<td>1</td>
<td>Never</td>
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<tr>
<td>2</td>
<td>Hardly ever</td>
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<tr>
<td>3</td>
<td>Occasionally</td>
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<td>4</td>
<td>Fairly often</td>
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<td>5</td>
<td>Very often</td>
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<td>8</td>
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Have you had any difficulty sleeping because of problems with your teeth and mouth?

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<tr>
<th>Rating</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Never</td>
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<tr>
<td>2</td>
<td>Hardly ever</td>
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<tr>
<td>3</td>
<td>Occasionally</td>
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<td>4</td>
<td>Fairly often</td>
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<td>5</td>
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<td>RF</td>
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Have you had any difficulty working because of problems with your teeth and mouth?

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<thead>
<tr>
<th>Rating</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Never</td>
</tr>
<tr>
<td>2</td>
<td>Hardly ever</td>
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<tr>
<td>3</td>
<td>Occasionally</td>
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<td>4</td>
<td>Fairly often</td>
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<td>5</td>
<td>Very often</td>
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<td>8</td>
<td>RF</td>
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Have you had any difficulty chewing because of problems with your teeth and mouth?

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Never</td>
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<tr>
<td>2</td>
<td>Hardly ever</td>
</tr>
<tr>
<td>3</td>
<td>Occasionally</td>
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<td>4</td>
<td>Fairly often</td>
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<tr>
<td>5</td>
<td>Very often</td>
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<tr>
<td>8</td>
<td>RF</td>
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<td>9</td>
<td>DK</td>
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</tbody>
</table>
Have you ever felt uncomfortable or embarrassed because of problems with your teeth and mouth?
1: Never
2: Hardly ever
3: Occasionally
4: Fairly often
5: Very often
8: RF
9: DK

Do you currently have insurance that covers all or part of your dental expenses?
1: Yes
2: No
8: RF
9: DK
If yes, is it:
1: An employer-sponsored plan
2: A provincial program for children or seniors
3: A private plan
4: A government program for social (welfare) clients?
5: A government program for First Nations or Inuit?
8: RF
9: DK

Finally, for our last question:
We’d like to estimate your total household income, the income received by all household members, from all sources, before taxes, for the past year. Was it:
   a. Less than $50,000
   b. More than $50,000
      If less than $50,000, was it?
         a. $10,000 to less than $15,000
b. $15,000 to less than $20,000  
c. $20,000 to less than $30,000  
d. $30,000 to less than $40,000  
e. 40,000 to less than $50,000  

If more than $50,000, was it?  

a. $50,000 to less than $60,000  
b. $60,000 to less than $70,000  
c. $70,000 to less than $80,000  
d. $80,000 to less than $90,000  
e. $90,000 to less than $100,000  
f. $100,000 to less than $150,000  
g. $150,000 and over  
h. DK/RF

*Thank you for taking the time to answer the questions. We can now proceed to oral examination.*
Appendix IV. Perceived Stress Questionnaire

Perceived stress scale

The Perceived Stress Scale (PSS) is one of the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one’s life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. The PSS was designed for use in community samples with at least a junior high school education. The items are easy to understand, and the response alternatives are simple to grasp. Moreover, the questions are of a general nature and hence are relatively free of content specific to any subpopulation group. The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way.

Evidence for Validity: Higher PSS scores were associated with (for example):

- Failure to quit smoking
- Failure among diabetics to control blood sugar levels
- Greater vulnerability to stressful life-event-elicited depressive symptoms
- More colds


Temporal Nature: Because levels of appraised stress should be influenced by daily hassles, major events, and changes in coping resources, predictive validity of the PSS is expected to fall off rapidly after four to eight weeks.

Scoring: PSS scores are obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 & 4 = 0) to the four positively stated items (items 4, 5, 7, & 8) and then summing across all scale items. A short 4 item scale can be made from questions 2, 4, 5 and 10 of the PSS 10 item scale.

Norm Groups: L. Harris Poll gathered information on 2,387 respondents in the U.S.
<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
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Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.
Name ___________________________ Date ____________
Age _______ Gender (Circle):  M    F    Other ______________________________

0 = Never  1 = Almost Never  2 = Sometimes  3 = Fairly Often  4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? ........................................ 0  1  2  3  4
2. In the last month, how often have you felt that you were unable to control the important things in your life? ........................................ 0  1  2  3  4
3. In the last month, how often have you felt nervous and "stressed"? .............. 0  1  2  3  4
4. In the last month, how often have you felt confident about your ability to handle your personal problems? ........................................ 0  1  2  3  4
5. In the last month, how often have you felt that things were going your way? ........................................ 0  1  2  3  4
6. In the last month, how often have you found that you could not cope with all the things that you had to do? ........................................ 0  1  2  3  4
7. In the last month, how often have you been able to control irritations in your life? ........................................ 0  1  2  3  4
8. In the last month, how often have you felt that you were on top of things? .... 0  1  2  3  4
9. In the last month, how often have you been angered because of things that were outside of your control? ........................................ 0  1  2  3  4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? ........................................ 0  1  2  3  4

Please feel free to use the Perceived Stress Scale for your research.

Mind Garden, Inc.
info@mindgarden.com
www.mindgarden.com

References
Appendix V. Financial Stress Questionnaire

Financial Stress Scale

These questions focus on your concern about your current financial situation compared to both the past and the future. Please circle the choice you think best fits your family situation.

1. During the past week, how often have you thought about any current or future money problems?
   a. Most of the time
   b. Often
   c. Occasionally
   d. Not at all

2. How much difficulty do you have in meeting the monthly payments of your household bills?
   a. Great deal
   b. Some difficulty
   c. A little
   d. No difficulty

3. In general, how do your finances usually work out at the end of the month? Do you usually have:
   a. Some money left over
   b. Just enough
   c. Not enough

4. Overall, how much do your finances stand in the way of your doing the things you want to do?
   a. Not at all
   b. A little
   c. Moderately
   d. A great deal

5. Do you have currently have dental insurance?
   a. Yes
   b. No

6. In the past year, have you avoided going to the dental professional because of the cost of dental care?
   a. Yes
   b. No

7. Is the home, apartment or condominium you live in:
   a. Currently owned by you, with mortgage already paid
   b. Currently owned by you, with mortgage currently being paid
   c. Rented
   d. Occupied without payment of money or rent
8. If you lost all your current source(s) of household income (your paycheck, public assistance, or other forms of income), how long could you continue to live at your current address and standard of living?
   a. Less than a month
   b. 1 to 2 months
   c. 3 to 6 months
   d. 7 to 12 months
   e. More than a year
Appendix VI. Honorarium Receipt

CONFIDENTIAL

RESEARCH PARTICIPANT RECEIPT FORM

The purpose of this form is to serve as documentation of the receipt of compensation associated with participation in a research study conducted at the Faculty of Dentistry, University of Toronto.

Research Participant’s Acknowledgement of

In appreciation of my involvement as a research participant,

I, ______________________________ (print name), hereby acknowledge receiving the amount of $ __________ from the University of Toronto.

I further acknowledge:

• that it is my responsibility to report the amount received for income tax purposes; and
• that the University of Toronto will not issue a tax receipt for the amount received.

Research Participant Signature                    Researcher signature

Date                                               Date