Polyamine Biomarkers as Indicators of Human Disease

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ABSTRACT

The significant increase of periodontitis, chronic kidney disease (CKD), Alzheimer's disease and cancer can be attributed to an ageing population. Each disease produces a range of biomarkers that can be indicative of disease onset and progression. Biomarkers are defined as cellular (intra/extracellular components and whole cells), biochemical (metabolites, ions and toxins) or molecular (nucleic acids, proteins and lipids) alterations which are measurable in biological media such as human tissues, cells or fluids. An interesting group of biomarkers that merit further investigation are the polyamines. The polyamines are a group of molecules consisting of cadaverine, putrescine, spermine and spermidine and these have been implicated in the development of a range of systemic disease, in part due to their production in periodontitis. Cadaverine and putrescine within the periodontal environment have demonstrated cell signalling interfering abilities, by way of leukocyte migration disruption. The polyamines spermine and spermidine in tumour cells have been shown to inhibit cellular apoptosis, effectively prolonging tumorigenesis and continuation of cancer within the host. Polyamine degradation products such as acrolein have been shown to exacerbate renal damage in CKD patients. Thus, the use of such molecules has merit to be utilised in the early indication of such diseases in patients.

Keywords: Biomarkers; Periodontitis; Polyamines; Chronic Kidney Disease; Alzheimer's Disease; Cancer.

1.1 Introduction

Periodontitis, chronic kidney disease, Alzheimer's disease, and cancer represent some of the most prevalent diseases within human populations. There is an urgent need to detect such diseases in their early stages, before they are able to progress and cause harm. The utilisation of disease specific biomarkers may provide valuable diagnostic information which can aid clinical decision making thus, increasing the potential of early interventions. Polyamines demonstrate a potential group of biomarkers which have been suggested to provide clinical evidence of active disease. Furthermore, oral diseases, namely periodontitis, have also been suggested to demonstrate significant correlations towards the incidence of systemic diseases (diseases which may be influenced due to transient bacteraemia and the distal deposition of oral microbial metabolites such as the polyamines) resulting in the metastatic spread of infection which induces systemic inflammatory reactions. Thus, the utilisation of polyamine based biomarkers in oral fluids may be a potential application that requires further exploration.

1.1.1 The oral cavity and its microenvironment

The oral cavity is of a unique design, whereby the juxtaposition of hard and soft tissues are continually exposed and challenged by numerous external material pressures (Taylor and Preshaw, 2016). It is one of the most complex regions on the human body and plays a vital role for providing entry, transit and exit for the digestive and respiratory systems, and it aids in the mastication and chemical pre-processing of foods (Yven et al., 2006). Within the oral cavity, there are hard tissues such as the teeth which are lined by the gingiva or the mucosa, and also soft tissue structures such as the cheeks, soft and hard palate and the tongue along with the periodontium, which is a collection of specialised connective tissues. The periodontium is a collective term which describes the tooth supporting and investing tissues such as the gingiva, root cementum, periodontal ligament and alveolar bone (Cho and Garant, 2000). This

significant number and variety of structures within the oral cavity allow for the colonisation of a substantial number of microorganisms on the oral surfaces.

In the oral cavity, there are two key biofluids present, these being saliva and gingival crevicular fluid (GCF). Saliva is a complex fluid which is secreted from three major glands in the mouth (90% total saliva production), along with a large quantity of minor glands (10% of total saliva production) (de Almeida et al., 2008). Secretion of the saliva from the salivary glands is a response mediated by the autonomic nervous system (Sarapur and Shilpashree, 2012).

1.1.2 Oral biofluids

The role of saliva is to protect the teeth, oral and peri-oral tissues, and to facilitate eating and speech (Dodds et al., 2015). Saliva is composed of a number of different electrolytes, including sodium, bicarbonates and phosphates, as well as immune complexes such as immunoglobulins, proteins and mucins (Humphrey and Williamson, 2001). The production of saliva is increased significantly upon the acknowledgment of food in the mouth through both mechanical and chemical stimuli (Neyraud et al., 2003). Both visual and olfactory stimuli have also been shown to increase saliva production (Keesman et al., 2016). In the oral cavity, saliva also acts as a lubricant, aiding in the initial digestion of foods through the formation of bolus for swallowing and initiation of the digestion of food components (Pedersen et al., 2002). Furthermore, due to the continual bathing of saliva in the mouth, this has shown to have a profound influence on the oral ecology and resulting microenvironment.

The GCF is an oral inflammatory exudate, which is derived from the periodontal tissues, and is found in the sulcus between the tooth and the gingiva (Lamster, 1997; Subbarao et al., 2019). Its role is to facilitate the antimicrobial defence of the periodontium and to maintain the structure of the junctional epithelium (Subbarao et al., 2019). Under normal

conditions, GCF is sourced in small volumes of 0.43 – 1.56 μL/h (Khurshid et al., 2017). However, the amount of GCF produced significantly increases, up to 44 μL/h in response to stimuli from the immune system and during periodontal disease. The constituents of GCF originate from the blood, surrounding cells and the various tissues of the periodontium (Lamster and Ahlo, 2007). Although the role of GCF is to prevent microbial mediated damage to the oral cavity, an increase in GCF has been shown to increase localised nutrients and provide a suitable physical environment for periodontal microorganisms, thus developing a positive feedback loop (Hickey et al., 2020).

1.1.3 Oral microbiome

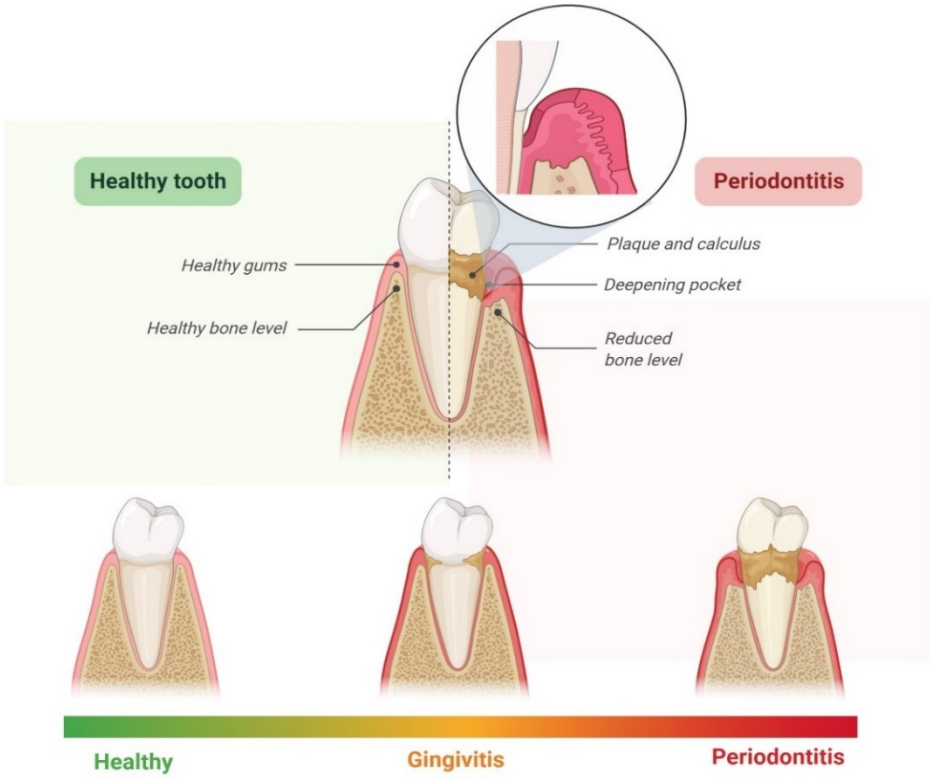
The oral microbiome is defined as the entire genome of microorganisms that reside within the oral cavity and it provides the second largest microbial community in humans (Deo and Deshmukh, 2019; Gao et al., 2018). It consists of a core microbiome and a variable microbiome. The core microbiome is common amongst all individuals, whereas the variable microbiome changes are dependent on the lifestyle of the individual and physiological pressures (Kilian et al., 2016). There are two types of surfaces which bacteria in the mouth are able to effectively colonise, the hard tissues such as the teeth, and soft tissues of the oral mucosa (Deo and Deshmukh, 2019). Once initial colonisation of a surface occurs, bacterial proliferation develops into a bacterial biofilm.

Comprised of over 700 diverse species of microorganisms, the oral cavity is a host for bacteria, fungi, mycoplasma and protozoa (Kuramitsu et al., 2007). The oral microbiota is imperative in the normal development of the host, effectively contributing to host defences, synthesis of important vitamins such as vitamin B and K and aiding in digestion. The oral microbiota is also involved in the prevention of exogenous pathogenic microorganisms, thus the relationship between host and oral microbiota is not one which is singularly passive (Patil

et al., 2013; Marsh, 2009). In terms of mutual and functional integration, the relationship and multifaceted balance between host and oral microenvironment determines the health status of the oral cavity (Cornejo Ulloa et al., 2019).

1.2.1 Periodontitis

Periodontitis is an orally, microbial driven inflammatory disease of the periodontium (Hajishengallis, 2015). The disease typically manifests as reoccurring inflammation of the gingiva, gingiva bleeding and the formation of periodontal pockets (Shatzle et al., 2004). It is estimated that between 20% – 50% of the global adult population are afflicted with periodontitis, making it the sixth most prevalent disease worldwide (Nazir, 2017). Periodontitis results in eventual loss of the periodontal ligament and subsequent destruction of alveolar bone by compromising the integrity of supporting tooth structures (Fig 1) (de Pablo et al., 2009; Al Moharib et al., 2014). From initiation, the disease progresses with the detachment of collagen fibres from the root cementum, apical migration of the junctional epithelium, deepened pocket formation and finally, resorption of the alveolar bone (Tsuchida et al., 2017). Periodontitis will ultimately progress to bone destruction if untreated, leading to increased tooth mobility with subsequent tooth loss (Hienz et al., 2015).



126

127 **Figure 1.** The decline of the healthy tooth and periodontium with the onset of gingivitis,

128 leading to periodontitis.

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1.2.2 Periodontitis and oral microorganisms

Dental bacterial biofilms (also known as plaques) are the main aetiological agents which causes gingival inflammation to progress to periodontitis (Hajishengallis, 2015). These dental plaques, associated with changes in the bacterial species, dysregulate the normal oral microbiota and as a result cause the inflammatory response of which periodontitis is indicative (Wahid et al., 2013). This increased microbial colonisation results in an increase in GCF flow, which anaerobic bacteria have been shown to favour. Anaerobic microorganisms which include *Porphyromonas gingivalis, Tanneralla forsythia* and *Treponema denticola* have been shown to be the most prevalent anaerobic bacteria associated with human periodontitis and they are frequently isolated in the majority of cases. These periodontopathogens alongside other "key accessory" microorganisms have been categorised into complexes based on their chronological colonisation of the gingival crevice and virulence in the subgingival plaques (Table 1).

Table 1. Subgingival bacterial classification in Socransky complexes (Socransky et al., 1998).

Bacterial Species	Complex
Streptococcus gordonii	Yellow
Streptococcus intermedius	
Streptococcus mitis	
Streptococcus sanguinis	
Campylobacter rectus	Orange
Fusobacterium nucleatum	
Peptostreptococcus micros	
Prevotella intermedia	
Actinobacillus actinomycetemcomitans	Red
Tannerella forsythia	
Porphyromonas gingivalis	
Treponema denticola	

The red complex bacteria encompass what is known to be the most pathogenic bacteria in human periodontal disease (Suzuki et al., 2013). These Gram negative bacteria become more prevalent during the later stages of dental biofilm development (Kesavalu et al., 2007). Studies have shown that there is an upwards of 96% infection rates of *P. gingivalis*, of which, 75% are in active periodontal sites, and 59.7% of *P. gingivalis* colonisation is found in inactive regions (Hernández et al., 2011; López, 2000). The colonisation of these areas by such microorganisms enables the initiation of inflammation to develop in the surrounding tissues, which can result in the loss of connective tissues and alveolar bone. This facilitates the conversion of the junctional epithelium to pocket epithelium (Bosshardt, 2018).

1.2.3 Dysbiosis in periodontal disease

Historically it has been thought that the red complex microorganisms were entirely responsible for the dysbiosis observed in the normal oral microflora through dysregulation of cell signalling pathways (Darveau et al., 2002). However, this understanding has since evolved, and it has now been suggested that periodontitis is a much more complex disease and is not solely regulated by one group of microorganisms. As a result, the descriptor of periodontitis infection is now based on the polymicrobial synergy and dysbiosis model (PSD) (Wang, 2015; Hajishengallis and Lamont, 2012). This model specifically refers to the communication between metabolically compatible microorganisms within an environment, which acquire functional specialisation through synergistic activities (Shaikh et al., 2018). The gingival crevice is colonised by an assembly of compatible microorganisms, grouped into heterotypic communities, to which the red complex bacteria and key accessory microorganisms interact. Such interactions increase community virulence and result in dysbiosis and tissue homeostasis disruption, causing the destruction of periodontal tissues (Hajishengallis and Lamont, 2012).

However, the bacteria at a specific region of the oral cavity change in relation to the tissues and structures to which they are bound and also to local differences in environmental pressures. An example of this occurs in fissures, on proximal tooth surfaces and in the gingival crevice, where two distinct variations of bacterial accumulations can be observed (Lamont and Hajishengallis, 2015).

The infection of the periodontal tissues presents a potential portal to enable periodontal microorganisms, bacterial metabolites and further biomarkers such as antigens into the body. Such bacteria and molecules may promote disease elsewhere in the body *via* dissemination of the circulatory system (Hickey et al., 2020). Such is evident in cases of Alzheimer's disease, and chronic kidney infections (Laugisch et al., 2018; Wahid et al., 2013).

2.1 The importance of biomarkers in human disease

A biomarker is defined as a biochemical, cellular or molecular alteration which is measurable in any biological media such as the human tissues, cells or fluids (Mayeux, 2004). Biomarkers are able to represent an objective indication of the medical state on an individual, and can be observed outside of the body, and measured with precision whilst maintaining a high degree of replicability (Strimbu and Tavel, 2010; Choong and Tsafnat, 2012). In the late 1990's, the National Institute of Biomarker Definitions working group described a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention' (Strimbu and Tavel, 2010). These biomarkers were henceforth divided into two distinct categories: exposure biomarkers, which enable the assessment of potential health risks, and disease biomarkers, which are currently in use for screening, diagnostics and monitoring existing diseases (Hwang et al., 2018; Strimbu and Tavel, 2010; Lowry, 1995). In order for a

biomarker to demonstrate a clear predictive value in which a disease or biochemical process can be effectively measured, definitive values for the biomarker need to be established.

2.1.1 Existing uses of biomarkers

In modern medicine, biomarkers are frequently utilised for screening of diseases such as Alzheimer's disease, cancer and periodontal diseases (Table 2) (Goossens et al., 2015; Sharma and Singh, 2016). Within such diseases, the uses of biomarkers may allow for confirmation of a diagnosis, initial detection of the suspected disease, or for monitoring the outcomes of theraputic interventions (Selleck et al., 2017). Biomarker research is now at the point where there is significant research input in the field of new biomarker discovery, and susequently, biomarkers are being constantly devleoped and refined. This has resulted in significant breakthroughs in the fields of drug discovery, clincal trials, epidemiology and personlised medicine (Collinson, 2013; White and Xie, 2013).

219 **Table 2.** Exisiting biomarkers used for the detection of current human diseases (Stathopoulou
220 et al., 2015; Lopez-Giacoman and Madero, 2015; Sauter, 2017; Sharma and Singh, 2016).

Alzhiemers disease	Cancer	Kidney disease	Periodntal disease
Tau protein	ctDNA (oral cancer)	Serum creatinine	Lypopolysaccarides
Phosporylated tau protein	Cytokeratin (in 90% of breast cancers)	Urinary albumin	Matrix metalloproteases
Amyloid B plaques	Ki-67 (cellular proliferation)	Cystatin C	Serum IgG

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222 For biomarkers in human disease to be effectively measured, they must be detected
223 within their respective biofluids. Current biofluids such as blood plasma, serum, urine and
224 cerebrospinal fluid are routinely used as a source of detecting biomarkers indicative of
225 disease (Xu and Veenstra, 2008; Kulic and Unschuld, 2016). The ability to effectively
226 replicate measurements of biomarkers to a high degree of accuracy and sensitivity has been at
227 the forefront of medical and epidemiological research (Thyagarajan et al., 2016; Tarnanas et
228 al., 2015). Within humans, the degree of biomarker variability between people results in a
229 range of acceptable 'normal' values. In such fluids, biomarkers are used as indicators of a
230 clinical manifiestation, disease stage, or to determine an alternate manifestation of the disease
231 in question (Mayeux, 2004). It is extremely important that the surrounding biofluid does not
232 interfere with the signal produced by the selected biomarker. Previous studies have shown

that although biomarkers may potentially have the capability to demonstrate levels which may be indicative of disease assocation, they may demontrate low levels of repeatability (Thyagarajan et al., 2016). Thus the reproducibility of biomarker measurements when used for for disease detection is still of concern (Yeh et al., 2017). However, it is evident that biomarkers potentially have signficant advantages to be used in disease detection over tradtional diagnostic methods although consideration needs to be given to the natural variability between human participants.

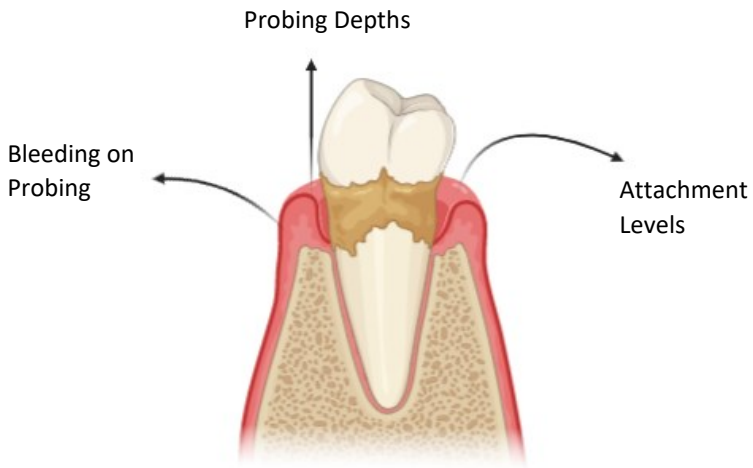
2.1.2 Infection biomarkers of bacterial driven disease

The use of biomarkers where typical diagnosis methods are insufficant, expensive and/or time consuming, may result in the most succesful application for the use of biomarkers (Lubell and Althaus, 2017). Since biomarkers are able to provide a clinican with valuable diagnositc and prognostic information regarding the current health staus of an individual, their applicability in various bacterially driven diseases is of major importance (Tang et al., 2017; Gomez et al., 2019). For example, tuberculosis (TB), is a communicable infectious disease that is known for having a long incubation time (2-8 weeks), resulting in severely delayed confirmation diagnosis. This is due to the detection methods relying on the bacteria being cultured to provide a positive confirmation. However, new biomarkers in the form of lipoarabinomannan, a virulence factor and glycolipid of the cell wall of the causative agent of TB (*Mycobacterium tuberculosis*) has shown good specificity to determine the presence of TB in the blood sptum and urine, without the use of bacterial cultures (Goletti et al., 2016; Correia-Neves et al., 2019; Wallis et al., 2010). This example demonstrates the effectiveness of the potential of new biomarkers as rapid detection alternatives. The use of such systems have the possiblity to reduce diagnosis times whilst maintaining comparable levels of diagnostic accuracy to that of traditional techniques.

3.1 Periodontal disease and its associated biomarkers

Periodontal disease is a prevalent disease afflicting a significant proportion of the human population, thus new rapid methods of detection and quantification that are non-invasive would be advantageous for use in patients rather than the use of traditional invasive interventions. Traditional methods of periodontal disease assessments make use of techniques such as bleeding on probing, pocket depth analysis, clinical attachment levels (CAL), plaque indexes, and analysis of radiographs of current alveolar bone levels (Fig 2) (Preshaw, 2015). These traditional measures are useful in the assessment of patient disease history but provide little to no information about the patient's current health, the active state of the disease, or risk of potential future periodontal breakdown (Srivastava et al., 2017). Furthermore, as the gingival pocket becomes more pronounced with disease progression, probing the area is painful for the patient. The use of a rapid biomarker detection system that relies on simply touching an area of the gum would provide a more rapid and less painful procedure for the patient.

The current aim for biomarkers in the field of periodontal research is to develop rapid, high impact diagnostics, which enhance clinical decision making. This should result in affordable, economically viable healthcare and an increase in favourable patient outcomes (Urdea et al., 2011). For such biomarker-based tests/measurements to become widely utilised, they must be at least of equal calibre to existing clinical diagnostics but show improvements in saving time, cost and also be easy to use for both the user and recipient.



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280 **Figure 2.** Clinical means of periodontal disease assessment utilising traditional methods of
281 bleeding on probing, probing depths and clinical attachment levels (Taba et al., 2005).

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284 *3.1.1 Saliva and GCF in periodontal disease*

285 It has been suggested that the saliva and GCF can be used to determine the periodontal
286 health status of an individual since the oral fluids contain specific disease biomarkers indicative
287 of periodontitis (Patil and Patil, 2011). Saliva which is readily available and collected without
288 invasive interventions provides an ideal source of periodontal biomarkers. Molecules are able
289 to be transported either into or out of the saliva and GCF through cells *via* passive diffusion,
290 active transport, or by extracellular ultra-filtration (Srivastava et al., 2017). When individuals
291 present with chronic inflammation, which accompanies periodontitis, the saliva becomes host
292 to a myriad of biomarkers. In recent years, the use of GCF has also gained wide interest as a
293 source of biomarkers for periodontal disease since it shows clear changes during different
294 stages of periodontal disease progression (Majeed et al., 2016; Ghallab, 2018; Barros et al.,
295 2016). Collection of GCF is a non-invasive procedure and it is simple to collect from
296 individuals with underlying comorbidities, since it is an inflammatory exudate which increases

in volume in response to a periodontal infection. Thus, both saliva and GCF appear to be attractive options for use as diagnostic fluids.

3.1.2 Biomolecules for periodontal disease detection

Periodontal disease can be attributed to the presence of certain bacteria species and as such, there has been numerous studies suggesting that the presence of antibodies directed towards the detection of these bacteria may implement new strategies for targeted biomarkers. In both the GCF and saliva of periodontitis patients, the presence of IgA and IgG antibodies have been sourced towards four periodontal disease specific bacteria, *P. gingivalis*, *A. actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium neucleatum*. Similar results between each bacterium were found, whereby a skewed pattern of significantly elevated levels of bacteria specific IgG and IgA antibodies were detected when compared to healthy controls (Plombas et al., 2002). Moreover, further studies compared 50 healthy individuals and 50 periodontitis afflicted patients. Significantly elevated levels of IgA and IgG antibodies respective to four keystone periodontal pathogens (*P. gingivalis*, *T. denticola*, *T. forsythia* and *A. actinomycetemcomitans*) were identified and demonstrated a periodontitis stage dependant increase in antibody concentration (Gadekar et al., 2018; Dye et al., 2009). Thus, IgA and IgG antibodies specific to keystone periodontitis pathogens in the GCF and to a lesser extent the saliva, are potentially able to indicate the potential 'at risk' sites for periodontitis due to their increased concentration (Takahashi et al., 1997).

The majority of damaging interactions which occur in periodontal disease take place at the crevicular and junctional epithelium (Fujita et al., 2018). Biomolecules, such as enzymes, endotoxins, nucleic acids, proteins, carbohydrates, degradation products and immunoglobulins, which result from periodontal bacteria residing in the GCF, have been shown to induce significant host tissue damage (Cekici et al., 2014; Barnes et al., 2014). Recent evidence would

suggest that the host inflammatory response to periodontal bacteria may also aggravate periodontal disease and demonstrate commonly identified disease specific pathologies (Nędzi-Góra et al., 2017). It has been demonstrated that under activation due to numerous chemical signalling molecules, polymorphonuclear leukocytes produce increasing levels of reactive oxygen species and proteolytic enzymes (Mariggiò et al., 2004). The hyperactivity of this response significantly contributes to the host tissue destruction during periods of active periodontal disease (Nair et al., 2014). During this host pathogen interaction many free amino acids and metabolites are significantly elevated (Barnes et al., 2009). Such elevation of potential biomarkers due to the mechanisms of infection mean that the whole saliva and GCF can provide a large, easily accessible pool of biomolecules for which periodontal disease detection and staging can potentially be evaluated. Further study is warranted in order to optimise the repeatability of measurements taken using such biomarkers and to determine how such markers are directly correlated with disease extent and progression.

4.1 Polyamines and periodontal disease

The colonisation of bacteria into gingival tissues is limited through salivary flow and muscular movements (Pedersen and Belstrøm, 2019). During a periodontal infection, the presence of GCF is increased and provides a more favourable environment for the formation of dental biofilms (Marsh, 2003). Over the course of infection, the composition of the sulcular dental biofilm composition shifts, from a mostly Gram positive and saccharolytic microflora, to a Gram negative, proteolytic microbiota (Berezow and Darveau, 2011). The Gram negative "keystone" bacteria are able to exacerbate the inflammatory events occurring in the periodontium though the release of biomolecules. One potential class of molecules, the polyamines, may be utilised as potential biomarkers to determine the incidence and severity of a periodontal infection.

4.1.1 Polyamine biosynthesis

The polyamines, cadaverine, putrescine, spermine and spermidine, are a group of organic polycationic molecules which are required for growth and differentiation of almost every eukaryotic and prokaryotic cell (Fig 3) (Takahashi and Kakehi, 2010; Shah and Swiatlo, 2008).

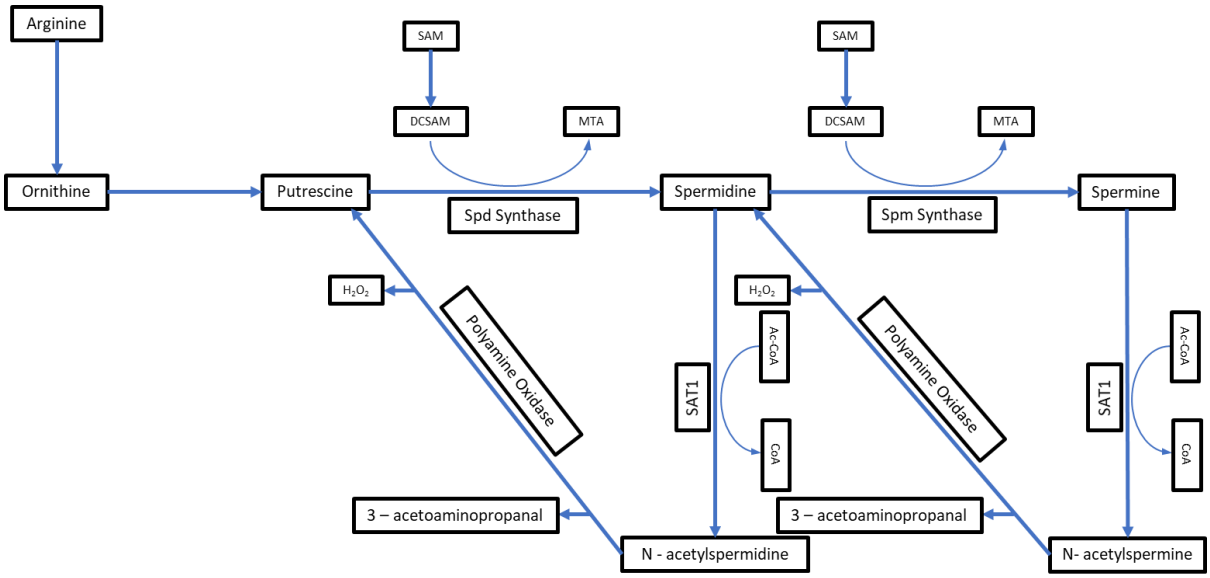

Figure 3. Biosynthetic pathways demonstrating the degradation of the biogenic amine, putrescine into its respective secondary and tertiary polyamines, spermidine and spermine.

These aliphatic polycationic molecules are ubiquitous in all tissues and cells in both plants and animals. Their roles within humans, span a broad range of functions, and these encompasses influencing cell apoptosis, division, differentiation, proliferation, DNA and protein synthesis, gene expression signal transduction and homeostasis (Fig 4) (Kusano et al., 2008; Pegg, 2016; Handa et al., 2018). Based on cellular distribution alone, putrescine and

spermine are the most prominent within human biosystems, aiding in cellular growth and division in both eukaryotic and prokaryotic cells (Handa et al., 2018).

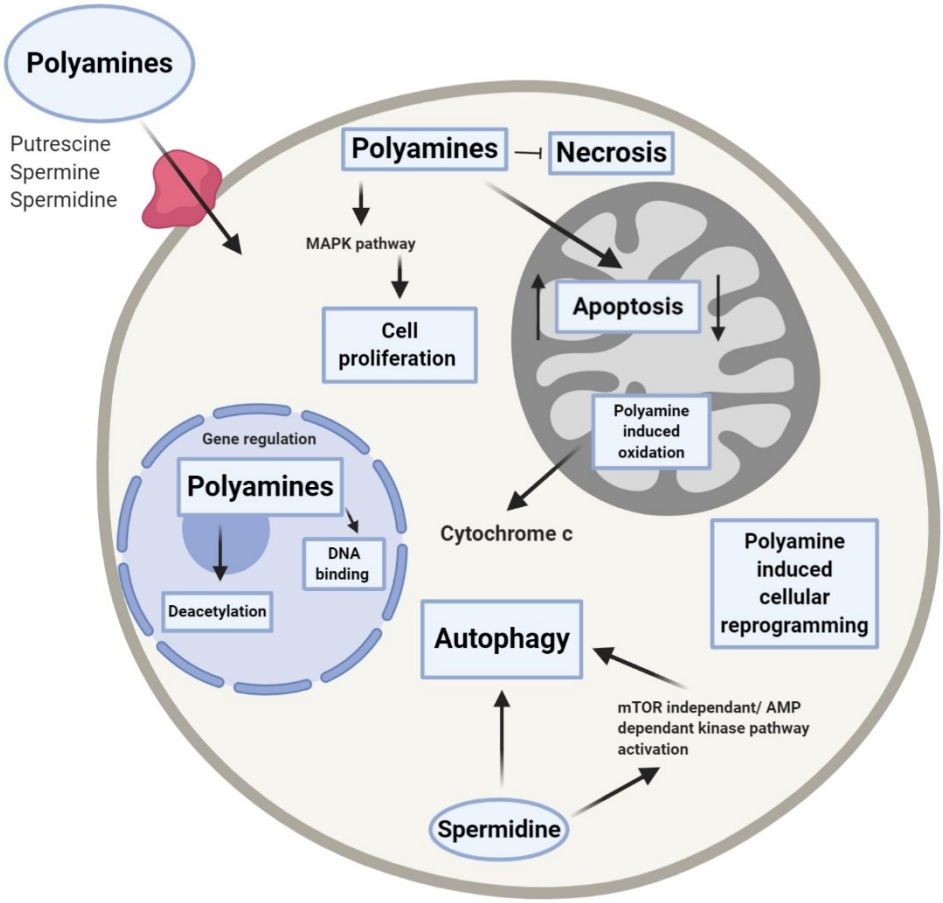

Figure 4. Polyamines express various functions upon entering the cell. The nucleus, mitochondria and cytoplasm are influenced differently, although, polyamines are a necessity for each cell component. Polyamines are involved in the regulation cell proliferation and termination, and also gene expression and translation. There is now increasing evidence to suggest that polyamines play a role in cellular reprogramming and autophagy.

374 *4.1.2 Polyamine in periodontal disease*

375 Cadaverine and putrescine have been identified as upregulated biomolecules in
376 periodontal disease and their role in the pathogenesis of periodontal disease has been suggested
377 in a number of previous studies (Lamster et al., 1987; Mariggiò et al., 2004; Lohinai et al.,
378 2012). Despite their abundance in cells, polyamine levels are tightly regulated. However, there
379 are some differences in the concentrations of certain polyamines such as cadaverine when
380 measured in the saliva of individuals. Tábi et al., (2008) measured average cadaverine levels
381 that increased to 11.8 ± 8.30 μM, from 7.9 ± 6.48 μM, after oral hygiene was restricted and
382 they concluded that such an increase in cadaverine concentration could potentially contribute
383 to an increase in the development of periodontal diseases. Fine and Mandel, (1986) described
384 increases of up to 10 fold of cadaverine in patients whose plaque index score was measured
385 from 1 to 2, leading to suggestions that cadaverine was the best indicator to measure the
386 metabolic activity of plaque which is associated with the onset of periodontal disease. The
387 upregulation of cadaverine has been observed by Lohinai et al (2012), through the
388 measurements of human dental biofilms. The concentrations of cadaverine, lysine and lysine
389 decarboxylase in dental biofilms after one week of oral hygiene restriction were measured as a
390 determination of damage to the gingival sulci. Cadaverine in this instance was produced as a
391 result of lysine decarboxylation and the cells attached to the dental tissues become lysine
392 deprived. This results in the release of pro-inflammatory cytokines, which act on the sub-
393 epithelial blood vessels to become permeabilised, or experience autophagy. This enables the
394 dental biofilm constituents to access the gingival stroma to release cytokines and initiate GCF
395 exudation.

396 The use of polyamines as potential biomarkers has been suggested in a number of
397 studies (Nakajima et al., 2018; Park and Igarashi, 2013; Sakanaka et al., 2017). Polyamines
398 such as putrescine and cadaverine, are frequently detected at increased levels in the GCF of

periodontitis afflicted individuals, which was made evident by Walters et al, (1987) whereby, 19 patients with mild to moderate periodontal disease measured a mean concentration of 921 μM and 615 μM for putrescine and cadaverine in the GCF respectively. The use of cadaverine specifically as a salivary biomarker for periodontal inflammatory status may be a possibility, since results have been shown to coincide with findings from previous metabolomics analyses of saliva from patients with predetermined periodontal disease (Barnes et al., 2014). The presence of cadaverine in human biofluid at greater than physiological concentrations is something typically introduced *via* an external source. Sakanaka et al (2017), postulated that cadaverine is actively metabolised by periodontal microorganisms due to significantly increased concentrations during periodontitis, thus its presence in saliva can be correlated to the number of bacteria colonising a specific region of the oral cavity. Furthermore, during metabolomics studies conducted using saliva from a periodontal patient, it was shown that a greater abundance of cadaverine was found in patients with an increased periodontally inflamed surface area (PISA), and the increase was attributed to putrefaction and breakdown of lysine via the oral bacteria.

Work by Lohinai et al., (2012), suggests that the dental biofilm site represents a significant location of cadaverine production. Since cadaverine is not typically present in healthy human blood, it has been suggested that the cadaverine from the dental biofilm diffuses from the site into the saliva, whereby it also penetrates tongue biofilms and the surrounding gingival sulci. In a case study by Lamster et al (1987), polyamine analysis of the GCF was conducted before and after two weeks of mucoperiosteal flap surgery on patients whom exhibited periodontitis. These areas demonstrated high values of putrescine pre-operatively (1020 pmol/mL). Similarly, putrescine values were observed at one week and two weeks post operatively (124 pmol/mL and 880 pmol/mL respectively). The polyamine content in the supragingival plaque of periodontitis afflicted individuals showed putrescine (1.49 nmol/mg)

was in the highest abundance, followed by spermine (0.03 nmol/mg) and spermidine (0.90 nmol/mg). This reduction in the concentration of spermine and spermidine was to be expected, since the direct degradation of putrescine leads to the formation of spermidine and spermine respectively (Pegg, 2016). The measure of putrescine in GCF provided better differentiation between relative periodontal health and diseased states since it was observed to be present at significantly higher levels in deep periodontal pocket sites in comparison to healthy controls (Ozeki et al., 2016; Lamster et al., 1987). Overall, it was suggested that the ability to quantify putrescine, spermine and spermidine within active periodontal sites was possible.

4.1.3 Polyamines in bacteria

Putrescine, cadaverine, spermidine and spermine are the predominant polyamines which are found in bacteria (Guerra et al., 2018). The role of bacterially derived polyamines has been shown to be associated with cell metabolism, cell-to-cell communication and bacterial cell differentiation, whilst also significantly contributing towards bacterial signalling, motility, and cell division (Kurihara et al., 2005; Igarashi and Kashiwagi, 2000; Miller-Fleming et al., 2015). Putrescine has also been determined to constitute to the outer membrane walls of some Gram negative bacteria such as *Salmonella enterica* and *Proteus mirabilis*, and cadaverine has been associated with the peptidoglycan of *Veillonella* spp. suggesting the importance of polyamines of maintaining the outer surface structures of bacteria (Shah and Swiatlo, 2008; Vinogradov and Perry, 2000; Kamio, 1987). Their synthesis relies on the presence of functional precursor molecules, similar to humans and these are detected at millimolar concentrations in bacteria (Tofalo et al., 2019). The intracellular concentrations of spermidine are determined to be the highest in bacteria at 1 – 3 mM, whilst putrescine demonstrate the lowest levels at 0.1 – 0.2 mM (Shah and Swiatlo, 2008).

448 Recent studies have investigated the impact of polyamines in bacteria (Goforth et al.,
449 2013; Nakamya et al., 2018). Polyamines are suggested to play a vital role in aiding the
450 pathogenesis of bacterial species. Cadaverine has been implicated in enhancing oral bacterial
451 proliferation through the inhibition of leukocytes thus decreasing the likelihood of bacterial
452 phagocytosis (Lohinai et al., 2012).

453 There have been several studies in which distinct pathways for specific virulence
454 mechanisms of bacterial species, (*Francisella*, *Legionella*, *Salmonella* spp. and *Shigella* spp.),
455 have shown that polyamines were an essential requirement to establish an infection (Jelsbak et
456 al., 2012; Nasrallah et al., 2011). Jelsbak et al (2012), demonstrated that by inhibiting
457 polyamine synthesis pathways in *Shigella* spp, the virulence was severely limited by reducing
458 bacterial ability to invade cells. This suggests that polyamines play a vital role in the
459 modulation of the virulence of bacterial pathogens.

460

461 ***5.1 Polyamines and inflammation in periodontal disease***

462 Inflammation is the physiological response to tissue damage which can result from
463 microbial infection, cellular damage or response to toxic compounds (Chen et al., 2018). In the
464 instance of acute inflammation, inflammatory cells such as leukocytes and lymphocytes are
465 attracted to the site of the inflammatory event through signalling networks of cytokines,
466 chemokines and growth factors (Babbar et al., 2007). Such cells contribute towards the
467 breakdowns of tissues, whilst also maintaining defence against infections.

468 Key microbial makers of metabolic origin, which may provide crucial information on
469 the periodontal inflammatory state of individuals, have been previously investigated as
470 potential biomarkers (Sakanaka et al., 2017). PISA has been used to investigate disease
471 associated metabolic signatures of periopathogens through a salivary metabolomics based
472 approach (Kuboniwa et al., 2016). The polyamine cadaverine, has been specifically identified

to positivity correlate with PISA levels. Work by others has demonstrated the efficacy of cadaverine at reducing the secretion of bactericidal superoxide enzymes, resulting in the disruption of host immune response signalling pathways which in turn reduces leukocyte migration to the site of inflammation (Kang et al., 2007; Lohinai et al., 2012).

Some studies have demonstrated that putrescine and spermidine, which have been isolated from the GCF and inflamed periodontal pockets at concentrations above 1 mM and 200 μM respectively, result in an increased disease stage of periodontitis. Furthermore, after treatment of the gingiva, the mean putrescine and spermine concentrations demonstrated significantly decreased concentrations. Such findings contribute to the theory of elevated polyamine levels in the GCF which coincide with increased periodontal disease severity for the patient. Polyamines in these instances are derived from oral bacteria and are released into the oral cavity following bacterial cell lysis as a response to the host immune system (Mariggiò et al., 2004). Polymorphonuclear leukocytes, a type of immune cell which is responsible for controlling inflamed gingival sites are influenced by bacterially derived polyamines, and as a result are forced to undergo apoptosis. This results in the continuation of gingival inflammatory processes, since these leukocytes continually migrate to the local gingival crevice in response to a chemotactic releases of plaque bacterium thus, contributing towards prolonging the effects and damage of periodontal disease (deHart et al., 2008; Mariggiò et al., 2004).

6.1 Polyamines and biomarkers of renal disease

Chronic kidney disease (CKD) is defined as the reduced kidney function that is present for three or more months and is determined using estimated glomerular filtration rate (GFR). The kidneys role is to filter the blood and metabolic waste whilst also altering the composition of fluids in the body. The loss off functional nephrons (basic structural unit of the kidney) results in a cascade of molecular and cellular events which may become pathological, and

develop into renal lesions (Rysz et al., 2017). Over recent years, CKD has become a major public health concern in both the developed and developing world (Fung and Kurella Tamura, 2016). CKD has a global affliction rate of up to 10% and is associated with a range of detrimental physiological and metabolic complications (Piccolli et al., 2017). It is characterised by the progressive destruction of the renal parenchyma, and along with the aforementioned loss of functional nephrons, ultimately leads to end stage renal failure (Viau et al., 2010). This progression is suggested to result from a perpetuating continuum of fibrosis, which remains active after the initial injury to the kidneys (Fogo, 2007). There have been numerous biomarkers identified for CKD, identifiable in a number of different locations and their effects on CKD have been suggested. Although CKD can be influenced by a range of conditions such as diabetes and high blood pressure and the effect of these comorbidities on CKD have been widely researched, much less research has been carried out to determine the influence of bacterially mediated progression of CKD (Thomas et al., 2008; Tuttle et al., 2019). Reported links between CKD and microorganisms isolated in distal locations have been studied, and one such correlation occurs between CKD and periodontitis (Lertpimonchai et al., 2019; Ariyamuthu et al., 2013). The potential systemic changes that periodontitis incurs are a result of dysbiosis of the oral microbiota, and the metastatic infection due to transient bacteria. In typical instances, the dissemination of oral microorganisms into the circulation result in no significant aftereffects other than short lived transient bacteraemia (Velzen et al., 1984). However, under favourable conditions the bacteria are able to localise and cause infection. The possibility of bacteria to undergo translocation from the oral cavity to distant body locations is one of the possible mechanisms in how oral microorganisms may contribute to systemic disease.

6.1.1 Biomarkers of chronic kidney disease

The estimation of kidney functionality has been previously conducted using biomarkers such as serum creatinine, elevated blood urea nitrogen levels and urine analysis (Rysz et al., 2017). However, biomarkers such as serum creatinine have been shown to possess low predictive values of CKD, thus efforts are being made into identifying new potential biomarkers for early CKD detection (Khan and Pandey, 2014). Within a clinical setting, GFR has been imperative in the diagnosis of CKD (Ruggenenti et al., 2012; Levey et al., 2014). GFR refers to the volume of fluid, which is filtered from the glomerular capillaries and into the Bowman's capsules as a function of time (Lopez-Giacoman and Madero, 2015). The GFR estimations are based on endogenous serum biomarkers such as serum creatinine or cystatin C. However, some studies suggest that biomarkers such as creatinine demonstrate suboptimal sensitivity and specificity in early CKD detection (Banda et al., 2020). In addition, factors such as age variations, sudden weight changes as well as current lack of standardisation of testing kits represent some of the issues with utilising serum creatinine as a CKD biomarker (Delanaye et al., 2014). Given the limitations of using serum creatinine as an indicator of kidney function decline, there has been an increase in the use of prediction equations using endogenous filtration biomarkers without the need for calculating renal clearance efficiency (Levey, 1990). Some studies suggest the effects of several alternate biomarkers for the detection and effective staging of CKD (Lopez-Giacoman and Madero, 2015).

6.1.2 Polyamines in chronic kidney disease

Since the 1970's, that the role of polyamines has been linked to CKD and it has been proposed that they may be influential in the pathogenesis of renal disorders (Macdougall, 2001). More recently, there has been an increase in the literature on the topic of polyamine-based CKD biomarkers (Igarashi et al., 2006; Saito et al., 1983; Sindhu, 2016; Goek et al.,

548 2013). Putrescine, a polyamine which is broken down into spermidine and spermine, was
549 shown to be significantly elevated in patients with chronic renal failure (Saito et al., 1983), and
550 it was postulated that such molecules may play an active role in the anaemia of end stage renal
551 disease (Table 3), since polyamines such as putrescine have been shown to reduce the
552 proliferation and maturation of erythroid cells (Kushner et al., 1991). Such studies suggest that
553 the measurement of polyamines such as putrescine, may aid in determining the health of the
554 kidneys in patients.
555
556 **Table 3**. The serum concentrations of putrescine, spermidine and spermine in normal and end
557 stage renal disease patients.

Pre and Post CKD levels of Serum Polyamines	
Normal Subjects	**Patients with ESRD**
Putrescine 0.24 nmol/mL	Putrescine 0.51 nmol/mL
Spermine 0.20 nmol/mL	Spermine 0.05 nmol/mL
Spermidine N/A	Spermidine 0.34 nm/mL

558

559

560 It has been demonstrated that the addition of spermine and spermidine to *in vitro*
561 cultures of human KB and bovine BME-UV1 cells causes significant inhibition of cell growth,
562 potentially due polyamine oxidation and breakdown into their respective components (Fusi et
563 al., 2008; Higgins et al., 1969). The concentrations of intracellular polyamines have been
564 shown to increase to millimolar levels, and during polyamine catabolism, the resultant H_2O_2
565 concentrations are sufficient to cause cellular oxidative damage (Murray-Stewart et al., 2016).
566 Furthermore, studies have demonstrated that there is an increase in the expression of polyamine
567 catabolic enzymes in distal organs, such as the liver in the event of kidney injury. Such
568 information supports the suggestion that disruption to the kidneys may have potential systemic
569 effects on distal organs via the upregulated metabolism of polyamines.

570

571 *6.1.3 Acrolein and polyamines as renal toxins*

572 In addition to the polyamines demonstrating toxic effects towards cell lines, a major
573 uremic toxin acrolein, derived from polyamine degradation, has been demonstrated to be in
574 high concentrations in those with chronic renal failure (Fig 5) (Sindhu, 2016). It was suggested
575 that the expressed levels of acrolein found in the patient plasma (170 μM/mL), were around 5
576 fold higher than in the plasma of healthy individuals (Igarashi et al., 2006). These
577 concentrations of may be sufficient to cause cell damage, which may in turn further progress
578 kidney disease. The levels of spermine/spermidine – N^1 – acetyltransferase (SSAT), an enzyme
579 which is essential for the breakdown of polyamines in serum, is also increased when the
580 kidneys are not functioning to their optimum capacity, such as in patients with kidney failure
581 or kidney injury (Zahedi et al., 2019). This action of polyamine degradation results in increased
582 levels of breakdown products which are rapidly excreted or oxidised by enzymes such as
583 putrescine oxidase, resulting in putrescine formation. This polyamine has been strongly

correlated with tissue damage in a large number of diseases (Minois et al., 2011). A rat perfusion model of ischemic reperfusion injury of the kidneys was used to measure the levels of putrescine, SSAT and putrescine oxidase. Such measurements demonstrated increased concentrations of upwards of 7.5 fold increases in the cortical collecting ducts of rat kidneys. The levels of SSAT peaked and remained unchanged after 12 h of reperfusion, indicating that the upregulation of SSAT can be utilised as a specific and sensitive biomarker for kidney injury (Zahedi et al., 2003). Thus, it has been hypothesised that the polyamines and their respective degradation enzymes may play a role in mediating kidney damage.

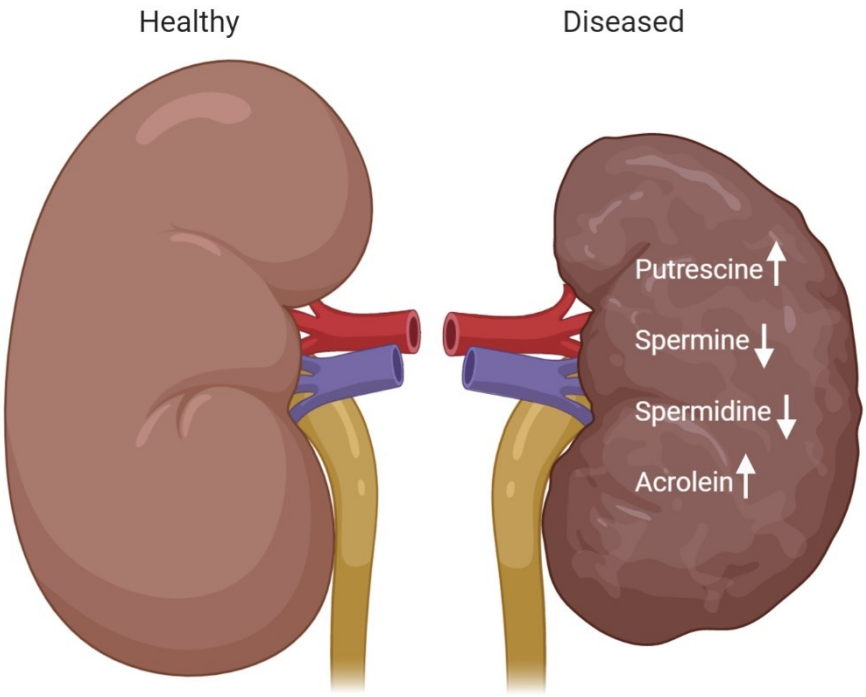

Figure 5. The changes in the polyamine levels as a result of chronic kidney disease. Putrescine levels are increase in kidney disease, which initially result in the increased formation of spermidine and spermidine. However, spermine and spermidine are rapidly broken down into

597 their oxidation products, namely acrolein. Thus, acrolein concentrations increase due to
598 increased breakdown of polyamine.

599

600 ***7.1 Polyamines as biomarkers of human cancer***

601 Cancer currently accounts for over one-sixth of global mortality rates (Bray et al.,
602 2018). Over recent years, the identification and application of cancer biomarkers has become
603 one the key foci of oncological research. Cancer biomarkers are described as biomolecules
604 which are produced by either tumour cells, or by cells in response to a tumour (Goossens et al.,
605 2015). Biomarkers of cancer must be able to provide prognostic, diagnostic or predictor values
606 for patient outcomes (Nair et al., 2018). Despite the efforts made in biomarker research (over
607 1000 individual markers discovered), only a small number of markers (<25) have been
608 approved for clinical use thus far (Vlachostergios and Faltas, 2019). A major issue is that
609 although some biomarkers may demonstrate strong clincal validation, their ability to decisively
610 contribute towards patient care is limited, outside of providing incremental, albeit clinically
611 non essential information (Diamandis, 2012).

612

613 *7.1.1 Polyamines as cancer biomarkers*

614 The use of polyamines in the detection of cancer has been studied throughout the
615 literature, since it has been suggested that their role in the pathogenesis of cancers such as
616 prostate, colon and pancreatic, is apparent (Nowotarski et al., 2013; Soda, 2011; Damiani and
617 Wallace, 2018). The first reported role of polyamines in cancer was documented by Russell
618 and Snyder, (1968), whereby the increased levels of ornithine decarboxylase (ODC), a
619 biosynthetic polyamine enzyme, was identified in various pathological cancers, such as liver
620 and breast cancer (Russell and Snyder, 1968; Deng et al., 2008). When supressed, the activity
621 of ODC, the rate limiting enzyme for mammalian polyamine biosynthesis, showed inhibition

of colon carcinogenesis in cancer rodent models. Such a finding may suggest that the reduction in polyamine biosynthesis demonstrated a positive correlation in reducing the rates of colon cancer in rodents (Erdman et al., 1999).

In cancer, polyamine metabolism is frequently dysregulated, and with overall polyamine levels increased significantly, and this has been suggested that they are a necessity for tumour progression and transformation (Murray-Stewart et al., 2016). It has been further shown that rapid tumour growth has been associated with polyamine biosynthesis, since polyamines are upregulated in actively growing tumour cells as demonstrated by Khuhawar and Qureshi (2001), whereby, plasma putrescine levels were increased from 0.97 µg/ mL to 1.65 µg/ mL after a single day of measurements. Furthermore, work has shown that inhibition of the enzymes which regulate polyamine systems have resulted in the regression of tumour growth (Russell, 1977; Khuhawar and Qureshi, 2001). Previous works have hypothesised on the use of polyamines as potential cancer biomarkers, but low marker sensitivity in tissues has been a hindrance in these instances (Casero et al., 2018).

In the blood, the increased levels of polyamines directly reflect the exacerbated levels of polyamine synthesis, which exist as a result of cancer tissues augmenting the synthetic abilities of amine oxidase enzymes (Sun et al., 2017). Such is evident in instances of breast cancer whereby, increased proliferation and progression of breast cancer, in part, is stimulated by overexpression of polyamine synthesis. Breast cancer is one of the most common malignancies which affects women, in the western world (Torre et al., 2017). ODC, a polyamine decarboxylating enzyme, has been detected at increased levels in breast cancer (2.42 nmol CO_2/ hr g), in comparison to benign tumours (0.62 nmol CO_2/ hr g) (Cañizares et al., 1999). This suggests that increased polyamine levels may contribute towards the active disease and therefore such biomarkers in the blood may allow valuable diagnostic measurements for the differentiation between active and inactive cancers. Furthermore, ODC has been recognised

as in independent prognostic factor for localised breast cancer, and successful attempts at blocking polyamine receptors on ODC have been shown to provide a beneficial impact on the implications of breast cancer treatment (Jun et al., 2007).

7.1.2 Polyamine upregulation and detection in cancer

The amino acid derived polyamines (putrescine, spermine, spermidine and cadaverine), have been acknowledged as metabolites which are a necessity for cellular growth, interacting with negatively charged molecules such as DNA, RNA and phospholipids to facilitate biological processes such as transcription, translation and differentiation (Gerner and Meyskens, 2004; Mandal et al., 2013). With such a vital role in key molecular processes, it is possible for complications to occur, suggesting that polyamines may also play a role in the advancement of malignant states and tumorigenesis of actively replicating cells (Battaglia et al., 2014). The biosynthesis of polyamines is an upregulated process in all actively replicating cells, of which includes cancerous cells and the baseline values for healthy versus disease patients have been determined (Table 4) (Erdman et al., 1999).

Table 4. Levels of polyamines in the urine of patients with active cancer, and normal controls.

	Putrescine (µg/ mg)	**Spermidine (µg/ mg)**	**Spermine (µg/ mg)**
Control	2.1	1.2	0.04
Solid tumours	3.7	2.7	0.6
Haematological tumours	4.4	3.7	0.8

Patients with enhanced polyamine concentrations of spermidine and spermine (45.15 nmol/10^{10} red blood cells and 17.27 nmol/10^{10} red blood cells in the instance of pancreatic cancer) in the blood have been shown to have more advanced disease states and more unfavourable prognosis, comparative to those with lower polyamine levels (15.04 nmol/10^{10} red blood cells, and 8.82 nmol/10^{10} red blood cells respectively), regardless of the type of malignancy (Uehara et al., 1980; Cipolla et al., 1994). Due to polyamines being vital for all cell growth, it is possible that increases in polyamine synthesis as a result of cancer could reflect enhanced tumour proliferation (Soda, 2011). Cancer cells with a greater capability to synthesize polyamines are associated with an increased production of biomolecules such as serine proteinase, matrix metalloproteinases, cathepsins and plasminogen activator, which can degrade the surrounding tissues (Mason and Joyce, 2013). Furthermore, increased cancerous tissues are able to produce elevated levels of vascular endothelial growth factors (VEGF), enabling sufficient blood flow to the cancer tissues, which in turn increases their uptake of polyamines, further augmenting cancerous cell migration (Auvinen et al., 1997; Ferrara, 2004). Cancerous cells invade surrounding tissues through migrations using blood vessel in order to establish secondary tumours (Soda, 2011). The overexpression of ODC and resultant increased polyamine synthesis is suggested to increase the invasive properties of cancerous cells therefore, it is possible that cancerous cells utilise polyamines as potential tumorigenesis enhancing molecules to progress its diseases state (Kubota et al., 1997).

7.1.3 Pancreatic cancer and its oral detection

Pancreatic cancer continues to maintain the worst prognoses with a five-year survival rate of only 9% (Ilic and Ilic, 2016). The poor prognosis rates alongside late presentation of pancreatic cancer demonstrates the importance of early detection, thus aiming to minimise the

patient's risk of progressing to later stages of the disease (Asai et al., 2018). Upon initial diagnosis, 30% of patients present with a locally advanced tumour and 50% are diagnosed with pre-metastasized disease (Canto et al., 2012). Throughout the literature, work on novel metabolite based biomarkers have been investigated for detection of early pancreatic cancer (Mayerle et al., 2018; Lindahl et al., 2017). Techniques such as capillary electrophoresis – mass spectrometry based metabolomics have been utilised to analyse metabolites within the saliva of pancreatic cancer patients (Asai et al., 2018). The results discovered 24 markers which enabled discrete differentiation of pancreatic cancer from other cancer types. Amongst the elevated concentrations of the discriminatory metabolites, the polyamines, spermine and N_1 -acetyl spermine were found to be specific to pancreatic cancer at their respective increased concentrations, thus presenting a possible option as a potential indicator of pancreatic cancer.

In summary, the utilisation of polyamines and their respective breakdown enzymes provide an insight into the mechanisms in which they influence various cancer pathologies. Furthermore, biomarkers such as polyamines are easily assessable in patients' saliva and urine, suggesting their potential use as a non-invasive cancer biomarker. Although these markers demonstrate a means of rapid and non-invasive detection for a variety of cancers, their specificity towards a singular cancer remains to be elucidated. Thus, such biomarkers may be utilised in an initial assessment as an early indicator of potential disease, prior to the consideration of more invasive means of testing.

8.1 Polyamines, periodontitis and Alzheimer's disease

Alzheimer's disease (AD) is currently the most common neurodegenerative disease in humans and is amongst the most difficult to diagnose and treat at early stages (McGhee et al., 2014). Population studies using the Delphi consensus method, have shown that AD prevalence exceeds 50% in subjects aged 85+ years, indicating that this is a significant disease in older

people (Ferri et al., 2005). AD is characterised in a clinical setting through progressive memory and orientation loss, which include various other cognitive reductions. As it currently stands, only symptomatic AD treatments exist, such as anticholinesterase inhibitors and antiglutamaterics (Aisen et al., 2012). These methods of treatment involve dealing with the symptoms of AD as they manifest and do not directly influence the current disease state. In order to inhibit disease progression there must be interference with the pathogenic stages of the disease, thus disrupting disease evolution (Yiannopoulou and Papageorgiou, 2013).

In current practice, the presence of AD is confirmed through measurements of amyloid beta protein, tau protein and phospho-tau levels in the patient's cerebrospinal fluid (Fig 6) (Sharma and Singh, 2016; Galimberti and Scarpini, 2010). Neurofibrillary tangles are a fundamental pathological hallmark of AD and are aggregates of hyperphosphorylated tau protein (Serrano-Pozo et al., 2011). Tau protein is a microtubule-binding protein which is mainly expressed in neurons (Stoothoff and Johnson, 2005). The hyperphosphorylation of this protein is what is thought to be one of the causes of tau dysfunction, causing binding to the microtubules in the neurons, leading to the eventual disruption of the neuronal microtubules with subsequent impairment of axoplasmic flow and loss of neuronal connectivity (Grundke-Iqbal et al., 1986; Iqbal et al., 2000; Blennow and Zetterberg, 2018). Studies have suggested that the pathological severity of AD is at its greatest during the early stages of the disease. Due to the invasive nature of current diagnosis and treatment strategies, research aims are focusing towards alternate biomarker sources thus, aiming at slowing the progression of neurodegeneration (Nelson and Tabet, 2015; Thalhauser and Komarova, 2012; Folch et al., 2016).

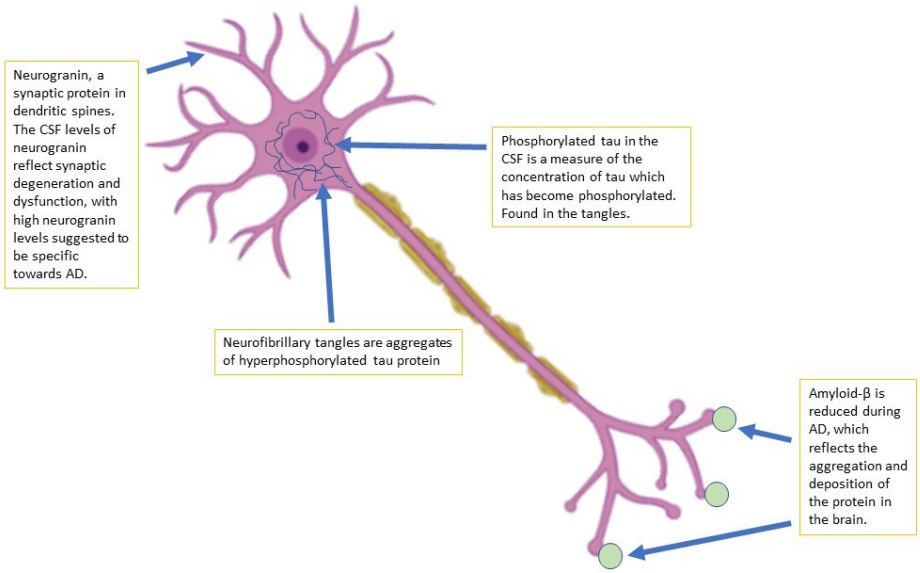

Figure 6. Schematic of the neuron displaying intracellular neurofibrillary tangles and neuritic amyloid plaques. Key biomarkers which hallmark Alzheimer's disease, and the synaptic neurogranin biomarkers are demonstrated in their respective boxes.

8.1.1 Biomarkers for Alzheimer's disease

As it currently stands, there is no disease modifying treatment available for AD, but trials for pre-symptomatic and early symptomatic stages of the disease continue to occur (Cummings et al., 2018). In recent works, Aducanumab, a monoclonal antibody, was cancelled during trails following evidence that it could not effectively slow the decline in cognitive function through amyloid beta production and aggregation (Panza et al., 2019). Therefore, the requirement for sensitive, diagnostic biomarkers for AD are essential, allowing for potential early disease modifying intervention.

There have been a number of suggested biomarkers for AD that have been discovered in the cerebrospinal fluid (Niemantsverdriet et al., 2017; Blennow, 2004). However, blood-based biomarkers have gained very little attention in the literature, with no blood-based

screening tool developed for use to this date (Panza et al., 2019). Blood-based biomarker presentation offers a distinct advantaged over conventional cerebrospinal fluid screens and neuroimaging modalities due to their advantages which include being cost effective, relatively less invasive and being able to provide an optimal starting location for further multistage assessment (O'Bryant et al., 2011). Due to recent advances in protein detection using mass spectrometry based approaches, there has been studies to define clinically relevant blood-based biomarkers (Long et al., 2016). Work by Ray et al (2007), identified 18 proteins in AD patient's plasma. These proteins allowed for differentiation between AD patients and healthy controls with a high specificity. Under further investigation, the same proteins were analysed, and it was shown that these proteins demonstrated diagnostic accuracy of 90% in identifying AD disease patients from healthy controls (Britschgi et al., 2011). With such findings, it may be possible to use blood-based biomarkers as predictors of AD. However, one issue arising from using this type of biomarkers is their specificity. Across multiple studies, large clinical cohorts were used in an attempt to identify markers of inflammation which are strongly associated with AD (Hu et al., 2012; Doecke et al., 2012; O'Bryant et al., 2011). Work carried out by the aforementioned researchers determined a high diagnostic accuracy with the 11 serum markers in test, including the polyamine cadaverine. The evidence suggested that cadaverine had some involvement in the inflammatory processes of AD, although there was no conclusive evidence of the exact role which cadaverine played. Therefore, the utilisation of new blood-based biomarkers has the potential to provide an alternative to conventional cerebral spinal fluid measurements.

8.1.2 Periodontitis influencing Alzheimer's disease

Periodontitis, due its potentially distal effects, has been suggested as major risk factor for the development of amyloid beta plaques and AD (Kamer et al., 2015). An observation

780 study consisting of 60 participants with mild to moderate AD was carried out by Ide et al
781 (2016), and over a six month period, with comparisons made between the oral health status of
782 the participant and cognitive decline. The presence of periodontal disease in these individuals
783 demonstrated a 6 fold increase in the rate of cognitive decline, suggesting a correlation between
784 the incidence of periodontal disease and the rate of cognitive decline in AD patients. In studies
785 conducted by Hill et al (2014), and Kitazawa et al (2005), mice whom overexpressed the
786 mutated tau precursor protein incurred significantly impaired cognitive function as a result of
787 a *P. gingivalis* mediated oral infection. Furthermore, an increase in the deposition of plaques
788 similar to those seen in AD patients demonstrated major oral bone loss. In addition to this, *P.*
789 *gingivalis* lipopolysaccharides (LPS) have been detected within the human AD afflicted brain
790 (Poole et al., 2013). These suggestions open up the hypothesis whereby, a *P. gingivalis*
791 infection of the periodontium may play a potential role in the pathogenesis of AD (Singhrao et
792 al., 2015).
793
794 ***9.1 Conclusion***
795 Periodontitis, CKD, cancer and AD are all diseases which afflict a significant portion
796 of the global population. Cadaverine represents a new potential biomarker for the detection of
797 periodontal disease, with its presence in oral biofluids being correlated with the extent of
798 periodontal tissue damage. It has been suggested for some time that there is a correlation
799 between periodontitis and CKD, whereby systemic locations are influenced by periodontal
800 bacteria or by their metabolites such as spermine, spermidine and acrolein, which have shown
801 significant detrimental effects towards kidney cells. The polyamine, putrescine has
802 demonstrated detrimental pathologies towards renal cells and has been suggested to act as
803 uremic toxin alongside its degradation product, acrolein. Thus, such biomarkers may aid in
804 current diagnostic strategies for CKD detection due to current markers such as creatinine

sometimes demonstrating low predictive values and significant variability between individuals. Spermidine and spermine demonstrate potential diagnostic biomarkers for the detection of cancer due to their significant involvement in the tumorigenesis of ongoing cancer pathologies. These polyamines are able to induce the production of vascular growth factors to enable increased blood flow to localised cancerous tissues. This in turn has suggested to increases polyamine uptake, augmenting cell proliferation and migration of oncogenic cells to distal sites. There is evidence to suggest the distal influences that periodontitis has on the systemic organs and tissues therefore, investigation into biomarkers which originate in the oral cavity could provide new means of disease detection. However, due to the limited number of studies investigating these associations in great depth, the evidence remains limited. Thus, further investigations are warranted to elucidate the reported interactions between these diseases.

The importance of polyamines in many molecular mechanisms in human disease is considerable. It is known that polyamines are in abundance in human biofluids during diseased states, and the evidence suggests strong correlations between the levels of polyamine and the extent or staging of a respective disease. Thus, it may be speculated that the utilisation of polyamine based biomarkers could provide the necessary diagnostic information in aiding efficient and rapid diagnosis of a disease.

For diseases such as periodontitis, traditional diagnostic means provide only information on the history of the disease, and not its current active state. Thus, a large amount of research is being undertaken to identify key biomarkers in oral fluids for not only oral infections, but also as a source of systemic diseases. Diseases such as prostate cancer is an example of a disease which effectively utilises biomarkers in their everyday screening and have shown a strong degree of functionality and repeatability. However, the uncertainty due to biomarker variability remains a challenge in the development of new biomarkers. Replicability and physiological variances between individual may hinder the effectiveness of a significant

portion of newly discovered biomarkers. Although these challenges do still remain, the utilisation of saliva and GCF as host media for biomarkers such as polyamines are very promising and warrant further investigation.

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Declaration of interest

No conflicts of interest were reported.

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