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Investigation of the possibilities for host modulation therapy for periodontal treatment

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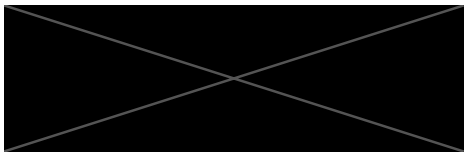
Author’s Declaration

I certify that the thesis presented here for examination for the degree of MRes of the University of Glasgow is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it) and that the thesis has not been edited by a third party beyond what is permitted by the University’s PGR Code of Practice.

Sridhar Rao



October 2025

COVID-19 Impact:

Recruitment challenges due to the COVID-19 pandemic

The clinical study component of this thesis faced significant recruitment challenges due to the COVID-19 pandemic, which imposed widespread restrictions on research activities across healthcare settings. This combined with the urgent reallocation of healthcare resources, led to the suspension or delay of many clinical research studies. NHS staff, including those in dental and osteoporosis clinics, were under exceptional pressure, with increased workloads and reduced capacity to support research activities. These operational constraints made it difficult to identify, screen, and recruit eligible participants. Compounding these difficulties, prescribing guidelines for denosumab were revised during the pandemic in response to emerging safety concerns, particularly the risk of rebound vertebral fractures upon discontinuation. As a result, the number of patients initiating denosumab therapy—who formed the target population for this study—dropped sharply. Ultimately, only one participant was recruited, highlighting the profound impact of both pandemic-related disruptions and regulatory changes on clinical research feasibility in this field.

Personal challenges due to the COVID-19 pandemic

I experienced multiple family bereavements (my mother in January 2021, my brother in April 2021, my father in May 2021, my mother-in-law in April 2022, and my father-in-law in October 2022). This resulted in a particularly challenging time during which I was working part time in clinics at Glasgow Dental Hospital and continuing with my Master's program. I am grateful to the Graduate School for extension to the Master's program to mitigate some of the disruption. Nonetheless, I have faced significant personal challenges which are further complicated by multiple and ongoing overseas travels to manage family affairs.

List of Figures

List of Tables

Table of Contents

Abstract

Background:

Periodontitis is a globally prevalent chronic inflammatory disease characterized by immune dysregulation and alveolar bone loss. Conventional therapies, while effective in many cases, often fail to achieve complete resolution, particularly in patients with systemic comorbidities or aggressive disease phenotypes. This has led to increasing interest in Host Modulation Therapy (HMT), which targets the underlying immunopathogenesis rather than the microbial component alone.

Aim:

This thesis investigates the potential of HMT in periodontal care, focusing on bone-modulating cytokines and the RANK/RANKL/OPG axis. It seeks to evaluate the feasibility of repurposing denosumab, a monoclonal antibody against RANKL used in osteoporosis, as a therapeutic adjunct in periodontitis.

Methods:

Two research questions were addressed. First, salivary concentrations of OPG, RANKL, and inflammatory cytokines (IL-1β, IL-6, IL-8, TNFα) were analysed in systemically healthy individuals with varying periodontal status (health, gingivitis, periodontitis) before and after treatment. Second, an exploratory clinical study assessed periodontal changes in a patients initiating denosumab therapy. Biomarker quantification was performed using multiplex immunoassays, and clinical inflammation was assessed using the Periodontal Inflamed Surface Area (PISA) metric.

Results:

OPG was variably detectable across health and periodontal disease states, while RANKL levels were consistently below detection thresholds. IL-1β showed a consistent reduction following treatment, suggesting its potential as a marker of therapeutic response. In the sample studied there were no significant correlations between cytokine levels and PISA. The denosumab study was limited by recruitment challenges and evolving prescribing guidelines, but provided valuable insights into the complexities of studying biologic agents in dental populations.

Conclusion:

HMT represents a promising adjunctive strategy in periodontal therapy, particularly for high-risk individuals. While current evidence supports the biological plausibility

of targeting host pathways, further research is needed to validate targets, optimise delivery systems, and establish long-term clinical efficacy. The integration of personalised medicine and biomarker-guided care may enhance the future role of HMT in periodontal practice.

Chapter 1: Introduction

1.1.1 Global Burden and Socioeconomic Impact of Periodontitis

Periodontitis is a prevalent and chronic inflammatory disease affecting the supporting structures of the teeth. It is among the most common non-communicable diseases worldwide, with an estimated global prevalence of over 50%, and severe periodontitis affecting up to 20% of adults, making it the sixth most prevalent disease globally (Tonetti et al., 2017: Trindade et al., 2023). The consequences of periodontitis include oral discomfort and tooth loss, as well as compromises to masticatory function, aesthetics, and psychosocial wellbeing.

In Europe and the United States, the combined direct and indirect costs of periodontal disease were estimated at €158.6 billion and $154.6 billion respectively (Botelho et al., 2022). These figures include productivity losses due to absenteeism, healthcare expenditures, and the impact on quality-adjusted life years (QALYs). Periodontitis accounts for a substantial proportion of all dental extractions, with up to 40% of extracted teeth removed due to periodontitis. Periodontal treatment can be effective but is time-consuming and resource intensive. Moreover, partial response to treatment and disease recurrence are common. Patients who undergo periodontal treatment require ongoing professional maintenance care and remain at risk of disease recurrence and tooth loss, contributing to long-term treatment demands and escalating costs.

1.1.2 Paradigms in Periodontal Disease Aetiopathogenesis

Historically, periodontitis was conceptualized as a plaque-induced disease, largely attributed to the direct effects of pathogenic bacteria residing in subgingival biofilms. This “specific plaque hypothesis” emphasized the virulence of species such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Tannerella forsythia* as primary drivers of tissue destruction.

Advances in microbial ecology and immunopathology have shifted this view towards a more integrated understanding. Current models recognize periodontitis as a disease of dysbiosis — a shift in the microbial community structure triggered and sustained by changes in host immunity (Hajishengallis and Lamont, 2021). The "Inflammation-Mediated Polymicrobial Emergence and Dysbiosis Exacerbation (IMPEDE)" hypothesis proposes that inflammation itself fosters an environment conducive to pathogenic microbial shifts, creating a self-sustaining cycle of host-driven tissue destruction. This paradigm acknowledges that microbial dysbiosis and immune dysfunction are intertwined, with inflammation not merely a consequence, but a driver of disease progression (Van Dyke, 2020).

1.1.3 Limitations of Current Conventional Therapies and the Need for Adjunctive Strategies

The gold standard of periodontal treatment—patient education, home-care instructions, risk factor management along with professional mechanical plaque removal, which may be supplemented with surgical intervention—is effective in many cases but has limitations. On average, even with gold standard treatment, 25% of sites within a patient's mouth fail to respond (Suvan et al., 2019). Sites with deep residual probing depths (≥6 mm), furcation involvement, or vertical bone defects often remain inflamed despite thorough treatment. Disease recurrence is common. Treatment responses are further compromised in patients with systemic comorbidities such as diabetes.

While antimicrobials have been employed as effective adjuncts, concerns about antimicrobial resistance, patient compliance, and systemic side effects limit their widespread long-term use. Additionally, these approaches primarily target the microbial component of disease while neglecting the central role of the host response. In this context, even optimal biofilm control may not prevent disease progression in susceptible individuals.

This has led to growing interest in therapeutic strategies that modulate the host immune-inflammatory response—either by downregulating destructive pathways or

promoting mechanisms of resolution and repair. These strategies fall under the umbrella of Host Modulation Therapy (HMT) and are referred to as HMT in the following sections.

1.1.4 Host Modulation Therapy (HMT) as a Targeted Biological Approach

Host Modulation Therapy refers to a range of pharmacologic and biologic interventions aimed at modifying the host response to reduce periodontal tissue destruction, promote resolution of inflammation, and support periodontal regeneration. Rather than simply suppressing inflammation, HMT seeks to more specifically target the underlying dysregulation of immune pathways that leads to damaging inflammation.

This potential value of HMT is highlighted by translational insights from other chronic inflammatory diseases such as rheumatoid arthritis, where biologic therapies targeting cytokines, immune cell receptors, and signalling pathways have achieved substantial clinical success. Using TNF as an exemplar, the translation of preclinical studies of TNF in RA resulted in decades of successful treatment of RA patients with TNF inhibitors (Feldman, 2002). Around two thirds of RA patients treated with a TNF inhibitor achieve a ‘moderate’ or ‘good’ response according to established criteria (Aaltonen, 2017). These seminal works cemented the use of TNF targeting agents in clinical practice, and extensive work investigating numerous other mediators now sees dozens of ‘biologics’ in routine clinical use for RA treatment (Smolen, 2022).

A further concept within HMT is resolution pharmacology—the use of pro-resolving lipid mediators such as resolvins, as pioneered by Van Dyke and Serhan (2008). These agents do not immunosuppress but actively orchestrate the cessation of inflammation, tissue healing, and return to homeostasis.

With accumulating preclinical and clinical data, HMT represents a paradigm shift in the management of periodontitis—from merely treating infection to actively restoring immune balance and protecting periodontal structure.

The following sections will explore the scientific foundations, therapeutic agents, clinical applications, and emerging frontiers of HMT in periodontal treatment, with consideration of mechanistic rationale, translational evidence, and the future role of HMTs.

1.2. Pathogenesis of Periodontitis: Rationale for Host Modulation

1.2.1 The Transition from Symbiosis to Dysbiosis

In health, the periodontal microbiome is maintained in a state of dynamic equilibrium with the host immune system. This symbiotic relationship promotes tissue homeostasis, immune surveillance, and resistance to overgrowth of pathogenic species. However, ecological disruption—driven by genetic predisposition, environmental stressors, or host immune dysfunction—can shift the microbial community toward a dysbiotic state. (Yamamoto, 2021: Abdulkareem, 2023).

In dysbiosis, the composition and function of the microbiota change, favouring organisms that can thrive in the inflammatory microenvironment and further exacerbate host response pathways. Periodontitis is thus not a classical infection, but a dysbiosis-driven inflammatory disorder in which the host's inability to restore microbial-immune homeostasis is fundamental (Abdulkareem, 2023).

1.2.2 The Host Response in Periodontitis

The host immune response in periodontitis involves coordinated activation of both innate and adaptive pathways (Baimi, 2025: Kinane, 2024). A full review of the roles of the innate and adaptive response is out with the scope of this introduction. Both are complex systems. Examples of highly studied features of the innate response are the pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which detect microbial-associated molecular patterns (MAMPs). Neutrophils are well established to play a key role in

periodontitis (Chapple, 2023). This leads to the release of pro-inflammatory cytokines (e.g., IL-1β, TNF-α, IL-6), chemokines, and matrix metalloproteinases (MMPs), which contribute to neutrophil recruitment and initial tissue destruction. A hallmark of periodontitis is an exaggerated and persistent cytokine response. IL-1β, TNF-α, and IL-6 are among the most studied consistently elevated cytokines in diseased periodontal tissues and gingival crevicular fluid (Preshaw, 2011). These mediators drive tissue destruction by promoting osteoclast activity, MMP expression, and neutrophil recruitment, creating a destructive inflammatory cascade.

The adaptive response is equally complex and extensively studied—although a full understanding of how antigen specificity relates to periodontal disease remains elusive. The biology of various T cell subsets has been studied in animal models and human tissues and led to some understanding of adaptive response biology in periodontitis. T-helper cells, particularly Th1 and Th17 subsets, produce cytokines such as IFN-γ and IL-17, sustaining inflammation and promoting osteoclastogenesis via the RANK/RANKL axis (Campbell, 2016). B cells also contribute by producing antibodies and modulating local inflammation (Berglundh, 2007). This immune activation becomes chronic and dysregulated, resulting in ongoing connective tissue breakdown and alveolar bone loss. Periodontitis is associated with a failure of regulatory pathways—such as T-regulatory (Treg) cells, IL-10 production, and the failure of activation of pro-resolving mediators—which should terminate the inflammatory response and restore homeostasis (Hathaway-Schrader, 2021). Resolution of inflammation is a highly orchestrated, active process involving specialized mediators that signal cessation of leukocyte recruitment, clearance of apoptotic cells, and restoration of tissue architecture (Van Dyke & Serhan, 2008). In health, inflammation resolves through the biosynthesis of specialized pro-resolving mediators (SPMs), including resolvins, protectins, and maresins. These lipid-derived mediators actively terminate inflammation by inhibiting neutrophil infiltration, enhancing efferocytosis by macrophages, and stimulating tissue repair (Balta, 2021).

1.2.3 Osteoimmunology: Inflammation-Driven Bone Loss

A central feature of periodontitis is the loss of alveolar bone, which ultimately compromises tooth retention. The biology of periodontal bone loss is increasingly understood within the framework of osteoimmunology, a field that explores the interaction between immune mediators and bone metabolism.

In periodontitis, cytokines such as Receptor Activator of nuclear factor K- ß Ligand (RANKL) (Lin J, 2014), Tumor Necrosis Factor alpha (TNF-α, commonly designated TNF) (Garlet, 2007), IL-1ß (Delima, 2002), IL-17 (Malcolm, 2015: Maekawa, 2015), and IL-33 have been shown in animal models to promote the differentiation and activation of osteoclasts, tipping the balance toward bone resorption. The RANK/RANKL/ osteoprotegerin (OPG) axis plays a pivotal role in this process. In healthy tissues, OPG acts as a decoy receptor to neutralize RANKL. In periodontitis, elevated levels of RANKL and decreased OPG expression shift the equilibrium in favour of osteoclastogenesis (Cochran, 2008).

Compared with individuals with healthy gingivae, periodontitis patients show elevated plasma concentrations of soluble RANKL (sRANKL), and reduced levels of OPG; suggesting that the ratio of RANKL to OPG will favour bone resorption more in periodontitis patients (Nile, 2013).

1.2.4 Systemic Implications of Unresolved Periodontal Inflammation

The effects of inflammation in the periodontal tissues are not confined to the oral cavity. Numerous studies have demonstrated associations between periodontitis and systemic conditions such as cardiovascular disease (Villoria, 2024), diabetes mellitus (Preshaw, 2019), rheumatoid arthritis (Lopez-Olivia, 2024), and adverse pregnancy outcomes (Kinane, 2008). Shared inflammatory mediators—including CRP, IL-6, and TNF-α may underlie these connections (Neurath, 2024: Villoria, 2024).

Persistent periodontal inflammation contributes to systemic inflammatory burden, possibly through translocation of bacteria or inflammatory mediators into the

circulation (Hajishengallis 2021). This low-grade systemic inflammation can exacerbate insulin resistance (Marruganti 2023), endothelial dysfunction (Teeuw 2014), and autoimmunity in susceptible individuals (Malcolm 2024). Other hypothesis linking periodontal inflammation with systemic effects include the alteration of trained innate immunity – patients with periodontitis have alterations in their bone marrow which manifest as long term changes to their innate immune cell functions (Hajishengallis 2022) .

There are shared risk factors, such as smoking, that will influence the relationship between periodontitis and systemic diseases. However, there is also the possibility that presentations of multimorbidity represent common underlying predispositions to deregulation of inflammation.

The systemic ramifications of unresolved periodontal inflammation provide a broader justification for HMT, especially in patients with comorbid conditions. By restoring immune balance locally, host modulation may help reduce systemic inflammatory load, adding a new dimension to the therapeutic impact of periodontal care.

1.3 Molecular and Cellular Targets in Host Modulation

The rationale for host modulation in periodontitis requires the identification of key molecular and cellular pathways that contribute to inflammation, tissue destruction, and bone resorption. These pathways include a wide range of cytokines, lipid mediators, complement, and signalling axes that govern immune cell behaviour and matrix turnover (Neurath, 2024).Understanding these targets enables the development of therapeutic strategies that either suppress destructive immune responses or support the resolution and repair of inflamed periodontal tissues. Some of the known ‘targetable’ pathways are described below.

1.3.1 The RANK/RANKL/OPG Axis

The balance between bone resorption and bone formation in the periodontium is tightly regulated by the RANK/RANKL/OPG signalling axis. Receptor activator of nuclear factor kappa-B ligand (RANKL) is expressed by osteoblasts, activated T cells, and other immune cells. When RANKL binds to its receptor RANK on osteoclast precursors, it promotes their maturation and activation. Osteoprotegerin (OPG), a soluble decoy receptor, produced by various cell types including osteoblasts, osteocytes, bone marrow stromal cells, periodontal ligament fibroblasts, gingival fibroblasts), B lymphocytes, activated T lymphocytes, dendritic cells, macrophages, and endothelial cells. Production of OPG is context dependent – for example dependent on the inflammatory state of the cells. OPG competes with RANK for RANKL binding and thereby limits osteoclastogenesis (Boyce, 2007: Yasuda, 2021).

In periodontitis, increased RANKL expression and reduced OPG levels create a local environment that favours bone resorption. The RANKL/OPG ratio in gingival crevicular fluid and tissues has been proposed as a biomarker of active bone loss (Caldeira, 2021) . Host modulation strategies targeting this pathway aim to restore the balance, directly inhibiting RANKL activity, and have been used in treatment of osteoporosis and cancers, for example multiple myelomas (Nayak, 2025: Gal, 2025). These systematic reviews and meta-analyses also document the side effects of such treatment which can include osteonecrosis of the jaw, and rebound bone loss on cessation of treatment.

1.3.2 TNF-α

Tumour necrosis factor-alpha (TNF-α) is a central pro-inflammatory cytokine involved in multiple stages of periodontal inflammation. It is produced by macrophages, T cells, and other immune cells in response to bacterial products and endogenous danger signals. TNF-α promotes the expression of adhesion molecules, enhances leukocyte recruitment, and stimulates the production of other inflammatory cytokines and MMPs (Preshaw, 2011).

High levels of TNF-α have been consistently detected in periodontal tissues and crevicular fluid from affected sites (Kadhiresan Rathinasamy, 2020). Systemic TNF-α inhibitors are used in the management of rheumatoid arthritis and other autoimmune diseases and there is ongoing interest in expanding the reach of TNF-α inhibition (Croft, 2024).

1.3.3 IL-1ß and IL-6

IL-1ß and IL-6 are involved in the amplification of inflammation and tissue breakdown. IL-1ß enhances the expression of MMPs and promotes osteoclast differentiation. IL-6 contributes to B cell maturation, Th17 cell differentiation, and the hepatic acute-phase response. Together, these cytokines play a synergistic role in sustaining inflammatory signalling in periodontal tissues (Arias-Bujanda, 2020).

Polymorphisms in IL-1 genes have been associated with increased susceptibility to periodontitis in certain populations (Karimbux, 2012), though the clinical utility of genetic screening remains uncertain. Therapeutic agents targeting IL-1 and IL-6 signalling, such as anakinra and tocilizumab, are approved for treatment of systemic inflammatory diseases including rheumatoid arthritis, juvenile idiopathic arthritis, and cytokine release syndrome (Petit, 2024).

1.3.4 IL-17 and the IL-23

IL-17, primarily produced by Th17 cells, has emerged as a key cytokine linking mucosal immunity to bone resorption (Gaffen, 2020). IL-17 promotes the recruitment of neutrophils, induces production of pro-inflammatory mediators by epithelial and stromal cells, and stimulates RANKL expression. Its upstream inducer, IL-23, supports the expansion and maintenance of the Th17 lineage (Gaffen, 2008).

Elevated IL-17 levels have been identified in periodontitis lesions, and experimental models suggest that IL-17 blockade can reduce bone loss (Maekawa,

2015). However, the dual role of IL-17 in maintaining mucosal barrier function complicates the therapeutic landscape, and there are case reports documenting how excessive inhibition may impair host defence in the oral cavity (Petit, 2024). IL-17 inhibition with secukinumab has been effective in psoriatic arthritis and other conditions including psoriasis and ankylosing spondylitis (Fragoulis, 2016).

1.3.5 Complement System

The complement system plays a key role in innate immunity and inflammation through its classical, alternative, and lectin pathways. Activation of complement component C3 generates effector molecules such as C3a and C5a, which promote leukocyte chemotaxis, cytokine production, and opsonization (Hajishengallis, 2016).

In periodontitis, local complement activation is thought to exacerbate inflammation and tissue injury. Experimental inhibition of C3 and C5a, for example using the peptide antagonist Cp40, has demonstrated reduction of bone loss and inflammation in animal models. Complement-targeted therapies offer a promising and increasingly well-characterized avenue for host modulation, with the advantage of intervening early in the inflammatory cascade (Hajishengallis, Hasturk, Lambris, 2021). Phase IIa trials in using a peptide inhibitor of C3 to manage gingival inflammation show promising results (Hasturk, 2021).

1.3.6 Pro-Resolving Lipid Mediators

Specialized pro-resolving mediators (SPMs), including resolvins, protectins, and maresins, are bioactive lipids derived from omega-3 fatty acids (Sahni, 2023). These mediators do not simply dampen inflammation but actively promote its resolution through processes such as inhibition of neutrophil infiltration, enhancement of macrophage efferocytosis, and stimulation of tissue repair. Significant reduction in inflammation and bone destruction has been reported in animal models (Hasturk, 2006). After several years of preclinical development, a

mouthwash formulation of a resolvin is now available (Hasturk, Schulte *et al,* 2021).

1.4 Principles and Mechanisms of Host Modulation Therapy

1.4.1 Definition, Aims, and Scope of HMT

Host Modulation Therapy (HMT) refers to the use of pharmacological or biological agents to modify the host immune-inflammatory response to periodontal pathogens. Rather than targeting bacteria directly, the goal of HMT is to interrupt the processes of inflammation and tissue breakdown, support the resolution phase, and promote tissue repair. By addressing the immunopathogenesis of periodontitis, HMT aims to improve treatment outcomes, reduce disease recurrence, and potentially limit the need for antimicrobials and / or more invasive therapies.

The scope of HMT includes both systemic and locally delivered therapies that influence cytokine activity, matrix degradation, osteoclast function, or inflammatory resolution. These may be used generally as adjuncts to conventional mechanical debridement or, in the future, as part of personalized treatment plans based on host response profiles.

1.4.2 Categories of Host Modulators

Host modulation agents can be broadly grouped into those that inhibit destructive pathways, those that promote pro-resolving pathways, and those that act through immune system recalibration. Therapeutic strategies are shown in Table 1.1.

Table 1.1 Summary of host modulation therapeutic strategies that may have relevance in periodontal disease.

Host-Modulation Strategy	Mechanism of Action	Approved Drugs (FDA / EMA)	Example Drug & Clinical Use
Enzyme inhibitors (MMP inhibitors)	Inhibit matrix metalloproteinases, preventing collagen and connective tissue breakdown	Subantimicrobial-dose doxycycline (SDD) approved for adjunctive periodontal therapy	**Periostat® (SDD)** – chronic periodontitis (Khattri, 2020)
Cytokine antagonists	Block pro-inflammatory cytokines (IL-1, IL-6, TNF-α), reducing inflammatory signalling	>20 approved: TNF-α inhibitors (~6), IL-1 blockers (~3), IL-6 blockers (~4)	**Adalimumab** (TNF-α inhibitor) – rheumatoid arthritis (Siebert, 2015)
Osteoclast inhibitors	Inhibit osteoclast-mediated bone resorption via RANKL or downstream signalling	At least 2 agents approved targeting this pathway	**Denosumab** (RANKL inhibitor) – osteoporosis, cancer-related bone loss (Bone, 2017: Nicolopoulos, 2023)
Pro-resolving lipid mediators	Promote physiological resolution of inflammation without immunosuppression	No resolvin-based drugs currently FDA/EMA approved	Experimental resolvins – early-phase human trials (Hasturk, Schulte *et al*, 2021)
Complement inhibitors	Inhibit upstream complement pathways involved in inflammation	~3–4 agents approved, mainly targeting C5 and related components	**Eculizumab** (C5 inhibitor) – paroxysmal nocturnal hemoglobinuria (Zhou, 2021)
Other immunomodulators	Small molecule immune modulators	Multiple agents e.g. JAK inhibitors	**Tofacitinib** (JAK inhibitor) –, rheumatoid arthritis (O'Neill, 2025)

1.4.3 Biomarkers for Disease Activity and Treatment Monitoring

Effective implementation of HMT is likely to requires tools to assess baseline disease activity, stratify patients according to those most likely to respond to a particular type of therapy, and monitoring therapeutic response. This is most likely to involve biomarkers in addition to clinical measures. Several molecular markers have been proposed as indicators of active disease or therapeutic efficacy, however none are yet validated for routine clinical use. Examples are shown in Table 1.2

Table 1.2 – Examples of potential biomarkers that may be useful in monitoring the impact of host modulation therapy in periodontitis.

Biomarker	**What it Reflects**	**Clinical / Biological Significance**
RANKL / OPG ratio	Balance between bone resorption and bone protection	Surrogate marker of osteoclast activity and alveolar bone resorption
IL-1ß, TNF-α, IL-6	Local inflammatory burden	Key pro-inflammatory cytokines involved in periodontal tissue destruction
Matrix metalloproteinases (e.g., MMP-8)	Collagen and extracellular matrix degradation	Associated with connective tissue breakdown in periodontitis (Rakic, 2025)
C-reactive protein (CRP)	Systemic inflammatory load	Links periodontal inflammation with systemic disease risk (Gupta, 2025)
Specialised pro-resolving mediators (SPMs)	Resolution capacity of inflammation	Potential biomarkers of effective inflammatory resolution (Lee, 2021)

Biomarker integration into clinical decision-making remains limited but is a growing area of research, particularly in the context of precision periodontal care. Salivary diagnostics have received significant attention – and in some cases are currently used in dental practice (https://www.bsperio.org.uk/patients/patient-

faqs-oral-microbiome-testing accessed 1st October 2025). However, these are not yet validated for integration into clinical care pathways. Nonetheless, these technologies are advancing at pace and it seems likely that in time biomarker-based point-of-care diagnostics and longitudinal profiling may eventually support more targeted and timely use of host modulators.

1.5. Clinical Applications of Host Modulation

Some pharmacologic agents have been investigated and approved for host modulation in periodontitis. The degree of clinical evidence supporting HMT in periodontitis varies, ranging from FDA-approved adjunctive therapies to experimental applications supported primarily by preclinical or off-label data. This section summarises key examples of host modulators in clinical or near-clinical use, with attention to their mechanisms, efficacy, and safety.

1.5.1 Subantimicrobial Dose Doxycycline (SDD)

Subantimicrobial-dose doxycycline (SDD) is the most established and widely studied host modulation agent in periodontal therapy. Administered at 20 mg twice daily, SDD inhibits matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-13, without exerting conventional antibacterial effects. The anti-collagenase action reduces tissue breakdown and supports healing following mechanical debridement.

Multiple randomised controlled trials and meta-analyses have shown that SDD, used adjunctively with scaling and root planing (SRP), results in statistically significant improvements in clinical attachment levels and reductions in probing depth. Benefits are particularly pronounced in patients with severe chronic periodontitis and in smokers and osteoporosis patients with reduction in the need for surgical treatment (Donos, 2020).

SDD is FDA-approved for chronic periodontitis and has demonstrated a favourable long-term safety profile. Although generally considered not to cause antibiotic

resistance, there are reports Periostat increases the number of antibiotic resistance species in the oral cavity (Haffajee, 2008).

Minor gastrointestinal side effects are occasionally reported but usually transient. Treatment is typically limited to 3–9 months, although longer durations have been studied.

Currently docycycline hyclate the active component of SDD is being used as a topical application in a mucoadhesive buccal film for prolonged release has also been suggested as an alternative (Dinte, 2023). Incorporated into nano spheres for local application has also been proposed (Lecio, 2020).

Although short term statistically significant benefits are reported, the long term clinical benefits with respect to hard outcomes such as tooth loss are not known (Herrera, 2020).

1.5.2 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs, including ibuprofen, flurbiprofen, and naproxen, have been investigated as modulators of periodontal inflammation via inhibition of cyclooxygenase (COX) enzymes and prostaglandin E2 (PGE2) synthesis. PGE2 is elevated in periodontitis and contributes to osteoclastogenesis and connective tissue breakdown (Gartenmann, 2020).

Short-term use of NSAIDs has shown reductions in gingival inflammation and alveolar bone loss in clinical and experimental studies. However, sustained benefits require continuous administration, and cessation is often followed by a rebound in disease activity in a study by Kurtis *et al* using 100mg of flurbiprofen twice daily for 10 days (Gartenmann, 2020).

Long-term use of systemic NSAIDs is limited by gastrointestinal, renal, and cardiovascular risks. Locally delivered formulations, such as flurbiprofen gel or ketorolac rinses, have been explored but are not currently in routine use.

Overall, NSAIDs offer proof-of-concept for inflammation targeting but are not recommended for routine HMT due to their systemic risk profile (Donos, 2020).

1.5.3 Omega-3 Fatty Acids and Specialized Pro-Resolving Mediators (SPMs)

Unlike traditional anti-inflammatories, SPMs—derived from omega-3 fatty acids such as eicosapentaeonic acid (EPA) and docosahexaenoic acid (DHA)—do not suppress inflammation but instead actively promote its resolution. This includes the cessation of neutrophil infiltration, enhancement of macrophage efferocytosis, and initiation of tissue repair mechanisms. Resolution is now recognised as a distinct and essential biological process.

Van Dyke and colleagues have been instrumental in advancing the therapeutic potential of resolvins, particularly Resolvin E1 (RvE1) (Sahni, 2023). In animal models of periodontitis, topical RvE1 reduced neutrophilic infiltration, restored bone levels, and promoted regeneration of the periodontal ligament and cementum.

While no resolvin-based therapies are yet approved, early-phase human studies have explored mouthwash and topical delivery systems, and safety data are encouraging. RvE1 analogues and synthetic SPM receptor agonists are also under investigation (Hasturk, Schulte *et al*, 2021).

Omega-3 fatty acid supplementation has shown clinical promise when used adjunctively with professional mechanical plaque removal (PMPR). Randomised trials report modest but consistent improvements in clinical attachment level, bleeding on probing, and inflammatory biomarkers. These effects are amplified when combined with low-dose aspirin, which triggers the biosynthesis of aspirin-triggered resolvins (AT-RvDs) (Sanz, 2020).

Doses and treatment durations vary across studies, and optimal regimens remain unclear. Nonetheless, omega-3 supplementation is generally safe, well-tolerated, and may be especially useful in patients with systemic inflammatory comorbidities.

However, there are risks associated with aspirin use, namely haemorrhage (Donos, 2020: Neprelyuk, 2023).

1.5.4 Statins

Statins are HMG-CoA reductase inhibitors with pleiotropic anti-inflammatory and bone-preserving effects. Locally delivered statins (e.g., simvastatin, atorvastatin) in gel or microsphere form have demonstrated improvements in periodontal parameters in small clinical trials. Mechanisms may include inhibition of RANKL-mediated osteoclastogenesis, stimulation of osteoblast differentiation, and MMP suppression (Rajagopal, 2022: Balta, 2021).

However, results are inconsistent, and heterogeneity in formulations and protocols limits generalisability. Systemic statin therapy, commonly used for cardiovascular risk reduction, may offer modest protective effects on periodontal status, though evidence is largely observational.

Statins remain a promising but unlicensed adjunct in periodontal therapy, requiring further standardisation and regulatory clarity (Donos 2020).

1.5.5 Probiotics and Nutraceuticals

Probiotics and various nutraceuticals have been investigated as adjunctive agents with host-modulating effects. Proposed mechanisms include immune modulation, anti-inflammatory activity, and restoration of oral microbial homeostasis.

Lactobacillus and Bifidobacterium species have shown reductions in gingival inflammation and periodontal pathogens in short-term studies. However, effects are often transient and vary by strain, formulation, and delivery method. Similarly, plant-derived compounds such as curcumin, green tea catechins, and resveratrol have been studied for anti-inflammatory properties, but clinical evidence is limited and inconsistent (Myneni, 2020: Sanz, 2020).

Overall, while generally safe, probiotics and nutraceuticals lack sufficient high-quality data for routine recommendation as host modulators. They may hold value in specific patient populations or as part of broader lifestyle interventions.

1.6 Biologic Host Modulators

"Biologics" are defined by the WHO as "a class of medicines which are grown and then purified from large-scale cell cultures of bacteria or yeast, or plant or animal cells. Biologicals are a diverse group of medicines which includes vaccines, growth factors, immune modulators, monoclonal antibodies, as well as products derived from human blood and plasma. What distinguishes biologicals from other medicines is that these are generally proteins purified from living culture systems or from blood, whereas other medicines are considered as 'small molecules' and are either made synthetically or purified from plants. Biologic therapies have transformed the management of systemic inflammatory diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Their mechanisms, targeting specific cytokines or immune pathways, align closely with the immunopathogenesis of periodontitis. While biologics have not been directly tested in periodontal patients, accumulating data from their use treating systemic conditions provides important insights into their potential relevance. Several cross-sectional studies have compared patients receiving different treatments for autoimmune and inflammatory diseases. Other longitudinal studies have monitored periodontal health following starting a biologic therapy. The majority of studies are small and confounded by polypharmacy and comorbidities and varying periodontal health at baseline. A scoping review identified several case reports of rare dental/oral side effects likely attributable to biologics including lichenoid reactions, angioedema and malignancy (World workshop ref France et al World Workshop on Oral Medicine VII: Oral adverse effects to biologic agents in patients with inflammatory disorders. A scoping review) Nonetheless, some evidence has emerged (Petit, 2024).

1.6.1 TNF-α Inhibitors

TNF-α is a central mediator of periodontal inflammation, and its inhibition has well-established efficacy in systemic diseases. Agents such as infliximab, etanercept, and adalimumab are widely used, for example in rheumatoid arthritis (Kerschbaumer, 2023), and Crohn's disease (Gorski, 2025). Case reports and small observational studies suggest that patients receiving systemic TNF-α inhibitors for arthritis may experience improvements in periodontal parameters, including reduced probing depth and gingival inflammation. Although some studies report increased bleeding on probing in the absence of any progression of periodontal attachment loss – implying a disconnect between tissue destruction and inflammation (Petit, 2024).

1.6.2 IL-1 and IL-6 Blockade

IL-1β and IL-6 are consistently implicated in periodontal tissue breakdown. **Anakinra** (IL-1 receptor antagonist) and **canakinumab** (anti–IL-1β monoclonal antibody) are approved for rheumatoid arthritis and autoinflammatory syndromes. **Tocilizumab** and **sarilumab** (IL-6 receptor inhibitors) are used in rheumatoid arthritis and giant cell arteritis (Sun, 2021: Balta, 2021).

Preclinical models indicate that IL-1 and IL-6 blockade can reduce periodontal inflammation and bone loss. In humans, indirect evidence from arthritis populations suggests possible periodontal benefit.

1.6.3 Janus Kinase (JAK) Inhibitors

Small-molecule JAK inhibitors (e.g., tofacitinib, baricitinib, upadacitinib) act downstream of multiple cytokines, including IL-6, IL-23, and interferons. They are approved for rheumatoid arthritis and Crohn's disease (Kerschbaumer, 2023: Gorski, 2025). Their oral administration and broad immunomodulatory profile raise

interest for potential local or systemic use in periodontitis. Small studies suggest these agents may have positive effects on periodontal parameters (Balta, 2021).

1.6.4 Anti-B Lymphocyte Therapies

B cells contribute to antibody production and cytokine release in periodontitis, and their dysregulation has been linked to tissue breakdown. **Rituximab**, an anti-CD20 monoclonal antibody, depletes B cells and is used in autoimmune conditions such as rheumatoid arthritis and systemic lupus erythematosus (Balta, 2021).

Indirect clinical observations suggest that rituximab therapy may attenuate periodontal inflammation in arthritis patients.

1.6.5 IL-17 and IL-23 Inhibitors

The IL-17/IL-23 axis is central to Th17-mediated inflammation and osteoclast activation. **Secukinumab** and **ixekizumab**(anti–IL-17A antibodies) and **ustekinumab** (anti–IL-12/23) are effective in psoriasis and psoriatic arthritis. Their mechanism suggests potential benefit in periodontitis, where IL-17 drives neutrophil recruitment and bone resorption (Neurath, 2024).

Animal models confirm that IL-17 blockade reduces alveolar bone loss (Maekawa, 2015: Gaffen, 2008). In human populations treated for psoriasis or arthritis, anecdotal periodontal improvements have been noted. The challenge remains balancing local periodontal benefit with the systemic need for intact mucosal immunity against fungi and bacteria – there are case reports of oral candidiasis following anti-IL-17 therapies and this is noted in the manufacturer's information (Neurath, 2024).

1.6.6 Complement C3 Inhibitors

Complement activation is a proximal driver of periodontal inflammation. Inhibition of C3 prevents generation of downstream effectors C3a, C5a, and the membrane attack complex. **Cp40**, a compstatin analogue, has shown efficacy in non-human primates, reducing inflammation and bone loss when applied locally (Balta, 2021)

Unlike other biologics, C3 inhibition is under evaluation in periodontal-specific clinical trials. This represents one of the most direct translational pathways from basic immunology to periodontal therapy. Early results are promising, suggesting that complement-targeted therapies may be among the first biologics to achieve regulatory approval for periodontal use (Balta,2021).

Systemic use of any biologic carries risks of infection and malignancy, limiting enthusiasm for their use outside of established systemic indications. Local delivery approaches remain speculative.

1.7 Bone-Modulating Agents in Periodontal Therapy

Alveolar bone loss is the defining feature of periodontitis and ultimately determines tooth prognosis. While inflammation drives this process, the biology of bone remodelling itself is a therapeutic target. Agents that inhibit osteoclast activity or promote osteoblast function have been explored as adjunctive strategies to protect or regenerate alveolar bone. Some of these drugs are already established in systemic conditions such as osteoporosis and metastatic bone disease, others remain experimental in periodontal applications.

1.7.1 Bisphosphonates

Bisphosphonates (e.g., alendronate, risedronate, zoledronic acid) inhibit osteoclast-mediated bone resorption by disrupting the mevalonate pathway. They are widely used in osteoporosis, Paget’s disease, and bone metastases. In

periodontal research, local delivery of bisphosphonates in gels or bone graft materials has been shown to enhance bone fill and clinical attachment gain in intrabony defects (Balta, 2021).

However, systemic bisphosphonate therapy carries a rare but serious risk of medication-related osteonecrosis of the jaw (MRONJ). For this reason, systemic administration is not appropriate for periodontal patients without another indication. Local delivery systems remain the most promising route but require further controlled trials to establish efficacy and safety (Balta, 2021).

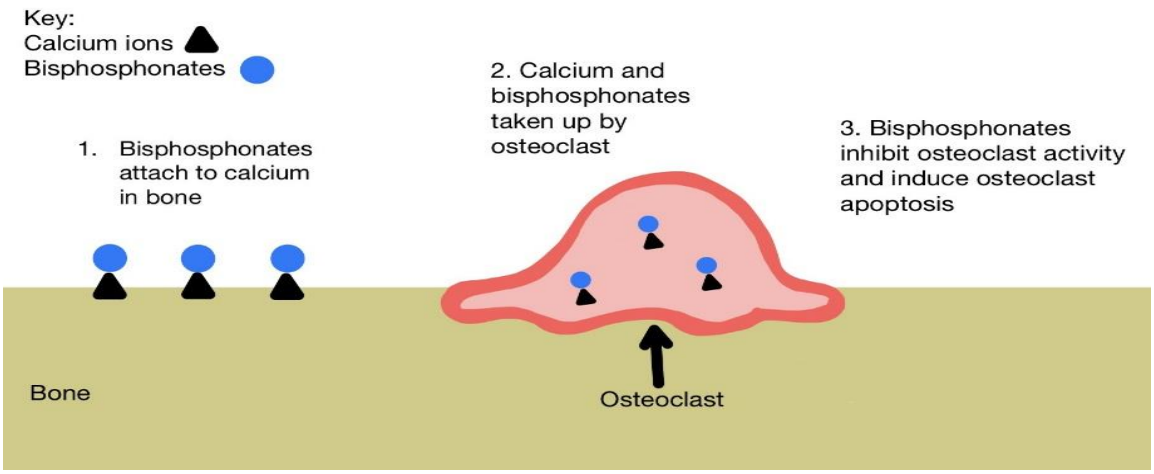


Figure 1.1: Schematic representation of Bisphosphonates mode of action on bone turnover.

1.7.2 Denosumab and OPG Mimetics

Denosumab is a monoclonal antibody that binds RANKL, preventing osteoclast differentiation and activation. It is approved for osteoporosis and prevention of skeletal-related events in cancer. Its mechanism directly targets the RANK/RANKL/OPG axis which is central to periodontal bone resorption. While

denosumab has not been systematically studied in periodontal therapy, its effects on alveolar bone are mechanistically relevant. Concerns parallel those of bisphosphonates, as denosumab also carries a risk - albeit small - of MRONJ. OPG mimetics, designed to replicate the natural decoy receptor function, have been developed experimentally but remain in preclinical phases. Denosumab has a much shorter half-life compared with bisphosphonates (Narayanan, 2013: Miku Kuritani, 2018).

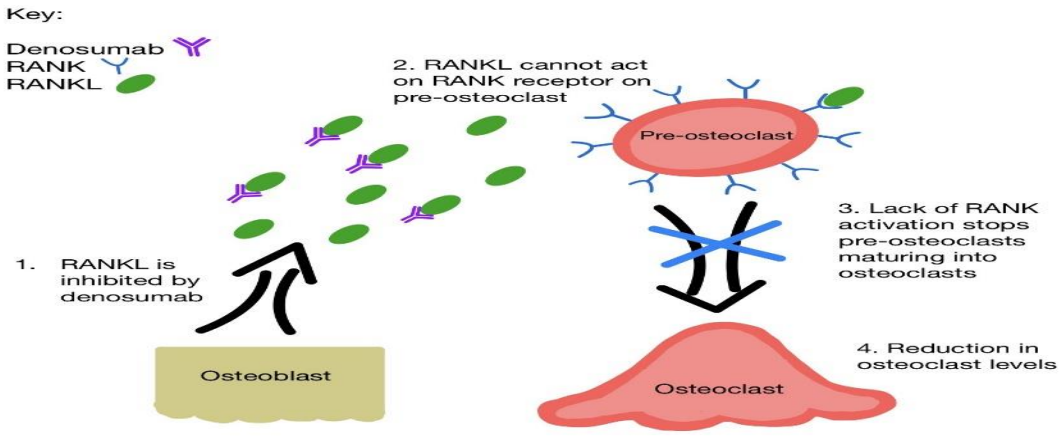


Figure 1.2: Schematic representation of action of denosumab on bone turnover

1.7.3 Bovine Lactoferrin and Natural Modulators

Lactoferrin, a naturally occurring iron-binding glycoprotein, exhibits antimicrobial, anti-inflammatory, and bone-modulating properties. Preclinical studies suggest that lactoferrin can promote osteoblast proliferation and inhibit osteoclastogenesis (Chen, 2021). In a human clinical studies a tablet containing lactoferrin and lactoperoxidase appeared to reduce gingival inflammation (Nakano, 2019). Lactoferrin has been associated with reduced inflammation and improved bone healing in animal models. Clinical evidence in humans remains limited, and larger trials are needed before translation into practice (Hou, 2012).

Other natural agents, including phytoestrogens and plant-derived polyphenols, have demonstrated osteoprotective effects *in vitro* and in animal models (Liu, 2022), but clinical data are insufficient to support therapeutic use.

1.7.4 Hormone Replacement Therapy (HRT) in Postmenopausal Women

Oestrogen deficiency accelerates alveolar bone loss and contributes to increased severity of periodontitis in postmenopausal women. Hormone replacement therapy (HRT) has been shown to reduce systemic bone resorption and improve bone density. Observational studies suggest that women on HRT may have lower rates of tooth loss and alveolar bone loss compared to untreated counterparts (Rahnama, 2013)

However, HRT is not prescribed for periodontal indications and carries systemic risks, including thromboembolism and certain cancers. Its role in periodontal therapy remains indirect—supporting the concept that systemic bone health and periodontal outcomes are interlinked (Rahnama, 2013).

1.8 Limitations of currently available evidence for HMT in periodontitis

While host modulation therapy (HMT) has generated substantial research interest, the clinical evidence base remains heterogeneous. A critical appraisal highlights both promising findings and significant limitations that must be addressed before widespread adoption.

Many HMT studies suffer from methodological weaknesses. Study design is complex for these trials. Except for the resolvins and complement inhibitors these have not yet been trialled purely for periodontal impact. Several studies involve patients with other inflammatory diseases who are taking host modulators for that condition. Therefore, studies can be cross sectional, comparing periodontal parameters in patients taking different medications; they can be longitudinal comparing patients at baseline and following a defined time of medication

treatment. A small number of studies provide periodontal treatment alongside the host modulation therapy. In all cases, study heterogeneity is challenging. Variability in patient populations, disease definitions, and treatment protocols makes comparison difficult. The studies are often single centre so the possibility to recruit large samples sizes is limited. Therefore, the studies are often exploratory with small sample sizes and limited statistical power.

Variability in endpoints recorded further confounds data interpretation. Clinical parameters such as probing depth reduction and clinical attachment level gain are standard but may not capture biological changes. There are no consistently accepted biomarkers that capture periodontal disease state/activity – so there is highly variable reporting of biomarker outcomes.

There is marked variability in trial duration. Most trials assess outcomes at 3–9 months, with limited evidence on long-term sustainability.

These limitations mean that while many studies report statistically significant improvements, clinical relevance and generalisability remain open to question.

Safety is a central concern. Some agents (e.g., SDD, omega-3 fatty acids) have excellent safety records, while others (e.g., NSAIDs, systemic biologics) carry risks that outweigh periodontal benefit in otherwise healthy individuals. Long-term surveillance data specific to periodontal patients are scarce. Post-marketing pharmacovigilance will be essential should new agents (e.g., complement inhibitors, resolvin analogues) reach clinical use.

HMT introduces potential additional costs to periodontal care. SDD and omega-3 supplements are relatively inexpensive, but biologics and complement inhibitors are costly and unlikely to be justified solely for periodontitis without systemic indications. Cost-effectiveness analyses are rare but will be crucial, especially in publicly funded healthcare systems. The broader argument—that improved periodontal stability may reduce systemic disease burden—remains attractive but requires stronger evidence.

Periodontal therapy is heavily dependent on patient adherence, and HMT adds complexity. Oral medications taken daily for extended periods may face compliance barriers. Acceptability varies, and nutraceuticals and supplements are generally well-received, while prolonged antibiotic-based regimens are a cause for concern. Patients often prioritise visible or symptomatic improvements, which may not align with the biological but less perceptible benefits of host modulation. Shared decision-making will therefore be key to effective implementation.

1.9. Potential integration of Host Modulation into Clinical Practice

The clinical adoption of host modulation therapy (HMT) requires clear guidance on where these interventions fit within the established framework of periodontal care. While professional mechanical plaque removal (PMPR), risk factor control, and supportive periodontal therapy remain foundational, host modulation has the potential to enhance outcomes in selected patient groups. Translation into practice involves considerations of treatment sequencing, patient selection, and long-term monitoring.

Periodontal therapy is typically structured in steps:

Step 1 includes risk factor management, personalised instructions in optimising home care, and supragingival professional mechanical plaque control. Step 2 further involves subgigival instrumentation. Step 3 can include periodontal surgery, and Step 4 involves maintenance treatment. HMT could be conceptualised as an adjunct in step 2 or step 3 (where antibiotics might currently be considered). In the maintenance phase, HMT may help sustain remission and reduce recurrence.

Figure 1.3 – schematic showing different steps of periodontal treatment.

The role of HMT is not likely to be uniform across all patients. Indications may include:

- **Persistent deep pockets or furcation involvement** despite adequate mechanical therapy,
- **High inflammatory burden** as evidenced by bleeding on probing, elevated biomarkers, or recurrent attachment loss,
- **Patients with aggressive or rapidly progressing disease phenotypes**,
- **Cases undergoing regenerative surgery**, where modulation of inflammation and bone resorption may improve outcomes.

HMT is an area where personalised medicine approaches may have significant benefit – for example it is likely that there are different drivers of periodontal inflammation in different patients, and these could be specifically targeted. Such approaches are emerging in treatment of other inflammatory diseases such as RA (REF)

Targeted use in these scenarios can maximise benefit while avoiding unnecessary intervention in low-risk patients.

A critical element of integrating HMT into practice is ongoing monitoring. Traditional measures (probing depth, bleeding on probing, radiographs) remain central, but adjunctive use of biomarkers may enhance precision. Salivary or crevicular assays for MMP-8, RANKL/OPG, or cytokines are not yet routine but represent a likely future direction.

Relapse prevention requires sustained host modulation in some patients. While long-term systemic use of biologics or NSAIDs is impractical, safer options such as periodic courses of SDD or continuous omega-3 supplementation may provide viable strategies for chronic care.

1.10 Future Directions and Research Priorities

Host modulation therapy (HMT) represents a paradigm shift in periodontal treatment, but its integration into clinical practice will depend on advances in mechanistic understanding, translational research, and precision implementation. Several key areas define the future research agenda.

1.10.1 Personalized Medicine and Host-Response Phenotyping

Periodontitis is not a uniform disease but a spectrum of host–microbe interactions shaped by genetic, epigenetic, and environmental factors. Precision medicine approaches—stratifying patients based on inflammatory phenotypes, genetic markers, or biomarker profiles—could identify those most likely to benefit from specific host modulators. Multi-omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, hold promise for developing such personalised treatment algorithms.

1.10.2 Integration of Systemic Biomarkers in Periodontal Care

The systemic connections between periodontitis and conditions such as diabetes, cardiovascular disease, and rheumatoid arthritis create opportunities to use shared biomarkers in clinical management. Incorporating systemic inflammatory markers (e.g., CRP, IL-6, HbA1c) into periodontal risk assessment could enhance case stratification and align dental and medical care pathways. Further longitudinal studies are needed to establish causal pathways and validate biomarker-based decision-making.

1.10.3 Combined Local and Systemic Modulation Strategies

Future therapies may combine local delivery of host modulators (e.g., gels, microspheres, or mouth rinses) with systemic interventions such as omega-3

supplementation. Localised delivery minimises systemic risk while achieving high drug concentrations at disease sites. Hybrid strategies could maximise efficacy, and minimise risks. However, local delivery systems are not optimised for periodontal treatment. Examples of currently local delivery systems are periochip and gels such as dentomycin. These have some efficacy but their applicability to local delivery of proteins remains challenging.

1.11 Knowledge Gaps

Host modulation therapy (HMT) represents a major conceptual and therapeutic advance in the management of periodontitis. Rather than focusing solely on microbial control, HMT addresses the dysregulated host response that underlies inflammation-driven tissue destruction. This shift reflects decades of progress in understanding periodontal pathogenesis, from dysbiosis and cytokine imbalance to the failure of inflammation resolution.

Evidence to date shows that certain agents—most notably subantimicrobial-dose doxycycline and omega-3 fatty acids—can safely and effectively enhance outcomes - albeit with small magnitude of changes - when used adjunctively with conventional therapy. Others, including NSAIDs, statins, probiotics, and locally delivered agents, offer proof of concept but require further validation. Biologic therapies, complement inhibitors, and pro-resolving lipid mediators illustrate the future direction of periodontal pharmacology, though most remain in translational or early clinical stages.

Critical gaps remain. The heterogeneity of trial designs with limited long-term data, and absence of validated biomarkers hinder routine clinical integration. Cost-effectiveness, patient adherence, and safety must also be addressed. Nonetheless, the rationale for host modulation is compelling, particularly in high-risk individuals and those with systemic comorbidities.

By combining local and systemic modulation with regenerative approaches, periodontal therapy can move beyond disease control toward true restoration of

health. Several gaps remain to be addressed before such step changes can be implemented.

1.12 Aims and research questions

This thesis aims to

i) investigate the role of bone modulating cytokines in periodontal disease and

ii) investigate the effects of denosumab on periodontal health.

Research Question 1

a. In patients who are systemically healthy with periodontitis, or in healthy volunteers with gingivitis or periodontal health: do salivary concentrations of OPG, RANKL relate to the clinical state of the periodontal tissues (health, gingivitis and periodontitis)?
b. In patients with periodontitis, do salivary cytokines (IL6, IL- 1ß,IL-8, TNF- α) associate with PISA at 0 and 90 days (before and 90 days after non-surgical (step 1 and 2) periodontal treatment)?

Research question 2

Do patients who start denosumab treatment for osteoporosis show evidence of changes in the oral cavity associated with altered periodontal health.

Chapter 2: An Investigation of mediators that may regulate bone turnover in patients with periodontitis

Hypothesis: In saliva of patients who have periodontitis, compared with patients who are periodontally healthy, there are differences in cytokine expression – in particular in cytokines associated with inflammation and bone resorption.

This chapter seeks to investigate **Research Question 1-**

a. In patients who are systemically healthy with periodontitis, or in healthy volunteers with gingivitis or periodontal health: do salivary concentrations of OPG, RANKL relate to the clinical state of the periodontal tissues (health, gingivitis and periodontitis)?

   Specifically, this question will be addressed by evaluating salivary concentrations of OPG, RANKL and how these relate to the clinical state of the periodontal tissues (health, gingivitis and periodontitis).

b. In patients with periodontitis, do salivary cytokines (IL6, IL- 1ß,IL-8, TNF- α) associate with PISA* at 0 and 90 days (before and 90 days after non-surgical (step 1 and 2) periodontal treatment)?

*see methods below for description of PISA

2.1 Materials and Methods

2.1.1 Sample Collection and Processing

Unstimulated whole saliva was used as the biological specimen. Approximately 5 mL of saliva was collected from each participant. Subjects were instructed to refrain from eating, drinking, or oral hygiene procedures for at least 1 hour prior to collection. Saliva was passively expectorated into sterile polypropylene tubes under supervision.

Immediately after collection, samples were placed on ice and transported to the laboratory. Saliva was centrifuged at 3,000 × g for 15 minutes at 4 °C to remove cellular debris. The clarified supernatant was aliquoted into sterile cryovials to avoid repeated freeze–thaw cycles and stored at –80 °C until analysis.

2.1.2 Study Cohorts

Periodontitis: Salivary samples were obtained from patients with periodontitis. These patients were part of a study "The **I**mmune **R**esponse **A**fter **P**eriodontal **T**reatment (IRAPT)" which was designed to measure local and systemic inflammatory changes following periodontal treatment. The study recruited patients between February 2018 and June 2019 and was a single-centre trial (Glasgow Dental Hospital). The study received ethical approval from the Office for Research Ethics Committees Northern Ireland (REC reference number: 18/NI/0059) and was conducted in accordance with the Declaration of Helsinki (7th revision, 2013) and the Research Governance Framework for Health and Community Care (2nd edition, 2006). Samples from a subset of patients were used in the current study. This subset was based on sample availability.

A full description of the complete cohort has been previously published (Johnston, 2020).

The PISA metric used throughout the analysis was calculated as previously described (Nesse, 2008). PISA was developed to reflect the surface area of bleeding pocket epithelium in square millimetres and thus provides an estimate of the local inflammatory burden posed by PD. The calculation of PISA builds upon an earlier metric (Hujoel et al., 2001) termed the 'dentogingival epithelial surface area' (DGES) which incorporates the CAL and data from a meta-analysis of root

surface areas to quantify the root surface area that has become exposed due to disease (Hujoel,1994). The periodontal epithelial surface area (PESA) expands on DGES to encompass PPD rather than CAL. Multiplication of PESA by the proportion of bleeding sites around a particular tooth generates PISA (Nesse et al., 2008).

Healthy controls (BPE 0, no loss of clinical attachment) and gingivitis (BPE 1 or 2 and no loss of clinical attachment) samples were obtained from healthy volunteers under the study "Host-microbiota interactions in oral health and disease", project number: 2011002. The healthy control study received ethical approval from the University of Glasgow MVLS ethical committee.
The clinical study of patients taking denosumab for osteoporosis is described in detail in chapter 4.

2.1.3 Biomarker Panel and Rationale

The primary analytes of interest were receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG), play a role in periodontal bone resorption. In addition, pro-inflammatory cytokines (IL-1ß, TNF-α, IL-6, and IL-8) which had been previously analysed in the samples, were used for comparative and correlative analyses. This strategy enabled evaluation of the broader immunological phenotype associated with periodontal inflammation.
The OPG and RANKL were analysed by the author in stored samples of saliva from all of the cohorts described above (healthy, gingivitis, periodontitis before and after treatment). Samples were thawed, and all analysed at the same time.
The analysis of IL-8, IL-1, IL-6 and TNF- α in the periodontitis patients were carried out by William Johnston and data shared with thanks. Salivary IL-8 was quantified using commercially available DuoSet sandwich ELISAs ((DY208-05 Biotechne – R&D systems, Abingdon, UK). Salivary IL-1, IL-6 and TNF- α were analysed by ELISA kits supplied by Thermofisher (IL-1: A35611; IL-6: BMS213HS; TNF- α: BMS223HS).
The analysis of IL-8, IL-1, IL-6 and TNF- α in the healthy and gingivitis saliva was carried using custom multiplex assay, R&D Systems, by Robert Reilly and data shared with thanks. Of note, this analysis confirmed that only IL-8 was detectable in the saliva of healthy or gingivitis participants.

Therefore, in this chapter, the data presented are the analysis of RANKL and OPG in ALL samples, and analysis of the salivary cytokines IL-8, IL-6, IL-1 and TNF- α in the periodontitis patients before and after treatment.

2.1.4 Laboratory Analysis (Luminex Assay)

Quantification of RANKL and OPG in saliva was performed using **ProcartaPlex™ multiplex immunoassay kits (Thermo Fisher Scientific, USA)**, based on Luminex xMAP® technology. The assay allows simultaneous detection of multiple analytes in small sample volumes with high sensitivity. The manufacturer's instructions were followed for all assays. Briefly, standards were made as instructed in the user manual. The Microparticle cocktail and Biotin-Antibody Cocktails were centrifuged for 30 seconds at 1000 x g and gently vortexed. Each cocktail was diluted in RD2-1 diluent. Streptavidin-PE was centrifuged for 30 seconds at 1000 x g, vortexed gently and diluted in wash buffer. To each well 50 µl of samples or standards were added, a further 5450 µl of diluted microparticle cocktail was then added, covered with the supplied foil plate sealer, and incubated for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) at 800 rpm. The plate was then washed. To wash the plate the 96 well plate was inserted into a magnetic plate holder, one minute after insertion 100 µl of wash buffer was added to each well, left for one minute before removing the liquid; the plate was washed three times. Next, 50 µl per well of diluted Biotin-Antibody Cocktail was added and a foil plate sealer was securely fitted, and the plate incubated for one hour at room temperature on the orbital shaker at 800 rpm. The plate was washed three times as described above. 50 µl of diluted Streptavidin-PE was added to each well and the plate was covered with a foil plate sealer, the plate was incubated at room temperature for 30 minutes on an orbital shaker set at 800 rpm. The plate was washed three times as previously described. The microparticles were resuspended in 100 µl of Wash Buffer and incubated for two minutes at room temperature on an orbital shaker at 800 rpm. The assays were analysed using a Bio-Plex 200 analyser (BioRad, Watford, UK). Analyte concentration was determined by interpolating the Mean Fluorescence Intensity (MFI) of samples against an asymmetric sigmoidal 5 parameter logistic standard curve. All standards and samples were conducted in technical duplicate. An outline of the process is shown in Figure 2.1.

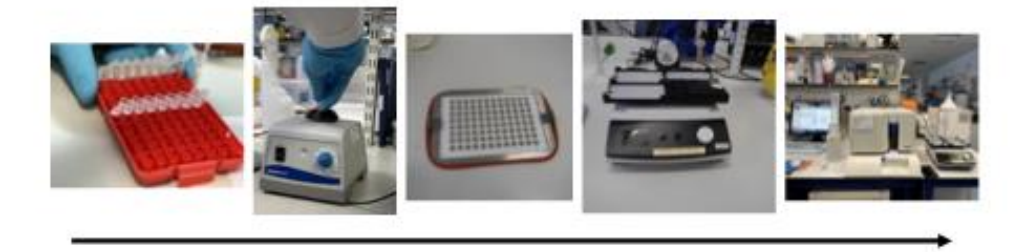


Figure 2.1 Photographic representation of Luminex workflow, showing (left to right): sample organising with appropriate diluent, mixing by vortimixer, magnetic plate, luminex specific plate shaker, and Luminex analyser.

2.1.5 Data Integration

For correlation of bone-related biomarkers with inflammatory mediators, RANKL and OPG levels were compared against cytokine, chemokine, and MMP profiles previously generated from the IRAPT cohort.

2.1.6 Statistical Analysis

All data were subject to cleaning whereby any unusual data points were checked against paper records. All variables were summarised using descriptive statistics (Minimum, Maximum, Median, Lower Quartile (Q1), Upper Quartile (Q3)). Due to the relatively small sample sizes, non-parametric statistical tests were undertaken. To test median differences between groups, Kruskal Wallis Tests were used. To test associations between continuous variables, Spearman's Rho correlation coefficients were calculated (with 95% Confidence Intervals).

2.2 Results

2.2.1 Participant Demographics

Samples from 14 patients with periodontitis were included, with samples from five volunteers with gingival health, and five volunteers with gingivitis. Table 2.1 presents the demographic and smoking status characteristics of the sample, according to periodontal status (health, gingivitis, periodontitis). The mean age of the healthy and gingivitis group was 32 and 29 years; with the periodontitis group the mean age was 49 years. Six of the periodontitis group were smokers. There were no smokers in the healthy / gingivitis groups. There were equal numbers of males and females in the periodontitis group; the healthy and gingivitis groups were both 80% male.

Table 2.1. Demographics of the sample

	Healthy (n=5)	Gingivitis (n=5)	Periodontitis (n=14)	All (n=24)
Gender				
Males	4	4	7	15
Females	1	1	7	9
Smoking Status				
Never	5	4	4	13
Former	0	1	4	5
Current	0	0	6	6

Age (years)					
	Min	Max	Median	Q1	Q3
Health (n=5)	30	39	32	30	35
Gingivitis (n=5)	25	53	29	26	35
Periodontitis (n=14)	33	54	49	39.25	49

2.2.2 Descriptive analysis of analytes

To evaluate distribution and range of data, the values for PISA and each of the cytokines measured were visualised. The baseline and Day 90 summary statistics for the sample and are shown in Table 2.2d.

Figure 2.2 shows the periodontal inflamed surface area (PISA) in the periodontitis group only. These data show baseline PISA between 600 and 2000 square mm. This is commensurate with severe periodontitis (Liera et al 2018). The PISA reduced significantly following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy) to less than 500 for all patients. After treatment, the majority of patients showed PISA of < 130 which is commensurate with mild disease or health. Therefore, these data show that the periodontal treatment effectively reduced clinical inflammation in the periodontitis group.

Figures 2.3, 2.4, 2.5, 2.6 and 2.7 show the individual data plots for each of the cytokines in the periodontitis patient group at baseline and Day 90. These data were used to provide an overview of the spread and distribution of the data for subsequent analysis. There were small, insignificant differences in cytokines pre and post treatment, with the exception of IL- ß which showed a marked reduction following treatment.

Several samples returned a ‘zero’ value for OPG (figure 2.3). Only one sample had detectable RANKL (data not shown as all other values were zero).

Table 2.2a Table depicting the salivary levels of IL-1 β in picograms/ml in health and gingivitis cohort

				Baseline		
	n	Min	Max	Median	Q1	Q3
Health	5	187.75	1187.7	614.1	367.74	1029.6
Gingivitis	5	309.8	869.5	713.9	364.8	753.17

Table 2.2b Table depicting the salivary levels of IL-6 in picograms/ml in health and gingivitis cohort

				Baseline		
	n	Min	Max	Median	Q1	Q3
Health	5	2.36	14.4	5.1	5	10
Gingivitis	5	2	8.2	3.4	2	7.5

Table 2.2c Table depicting the salivary levels of TNF-α in picograms/ml in health and gingivitis cohort

				Baseline		
	n	Min	Max	Median	Q1	Q3
Health	5	1.8	25.41	2.8	2.4	8.9
Gingivitis	5	0	8.3	2.8	1.5	5.6

Table 2.2d. Baseline and Day 90 values (periodontitis participants only)

		Baseline					Day 90				
	n	Min	Max	Median	Q1	Q3	Min	Max	Median	Q1	Q3
PISA (mm^2)	14	684.9	1969.7	1082.4	910.1	1614.21	18	400.3	163.6	79.8	277.9
OPG (pg/ml)	14	0	172.7	63.92	5.001	123.9	0	285.4	13.6	0	103.1
RANKL (pg/ml)	14	0	0	0	0	0	0	0	0	0	0
IL6 (pg/ml)	14	0.92	53.3	3.66	3.1	7.3	1	11	3.5	2.3	5.6
IL8 (pg/ml)	14	256.6	1909	430.1	309.4	889	121.8	1520.5	438.3	181.4	504.7
IL1B (pg/ml)	14	176.9	1890.6	395.14	278.23	683.9	320.2	1125.2	182.4	144.8	391.2
TNF-α (pg/ml)	14	0.8	30.42	4.7	2.56	11.4	0.1	17	1.9	1.5	5.04

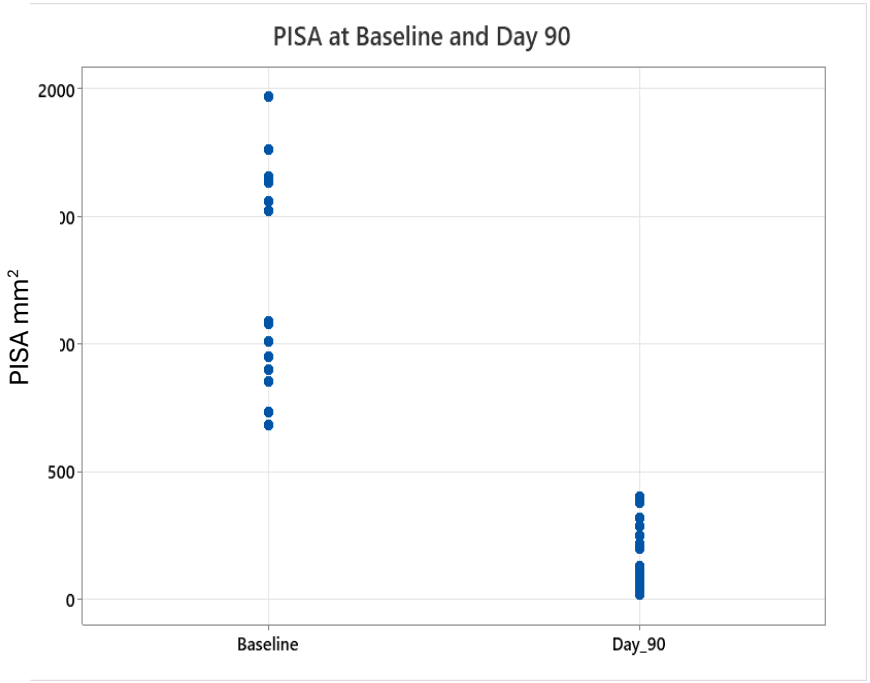


Figure 2.2: PISA at Baseline and Day 90
Periodontal inflamed surface area in patients with periodontitis at baseline (before non-surgical periodontal treatment) and 90 days after non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy).

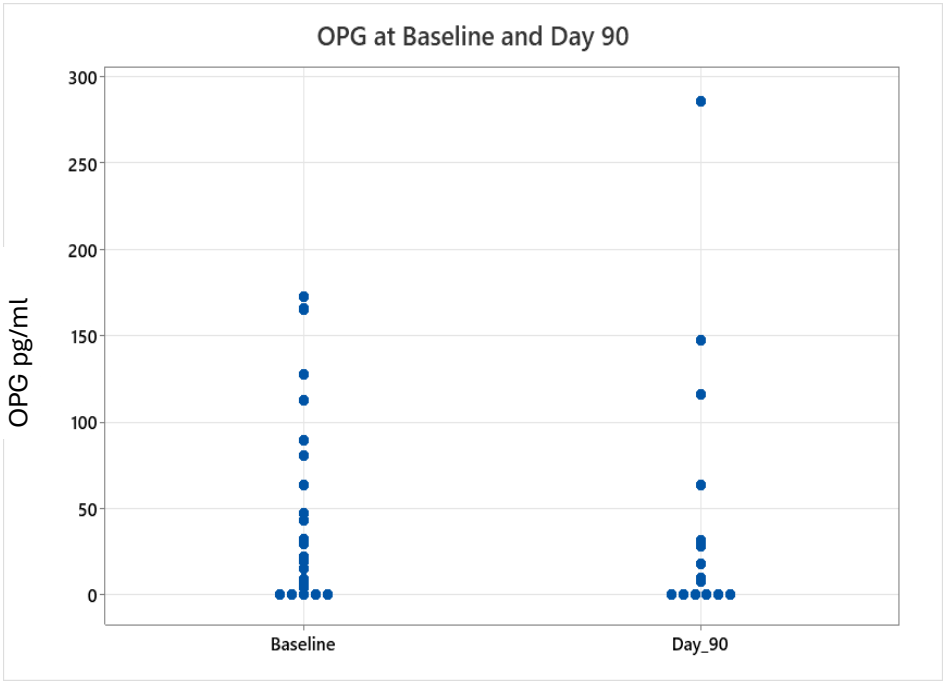


Figure 2.3: OPG at Baseline and Day 90
Osteoprotegerin, measured by Luminex in saliva, at baseline and following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)treatment in patients with periodontitis

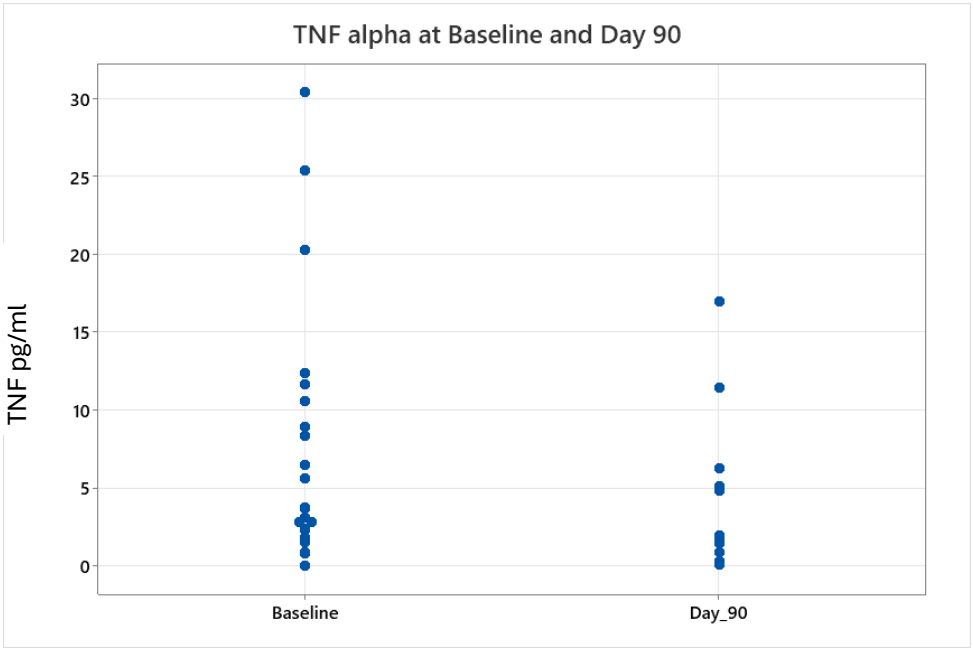


Figure 2.4: TNF- α at Baseline and Day 90
TNF- α, measured by Luminex in saliva, at baseline and following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy) in patients with periodontitis

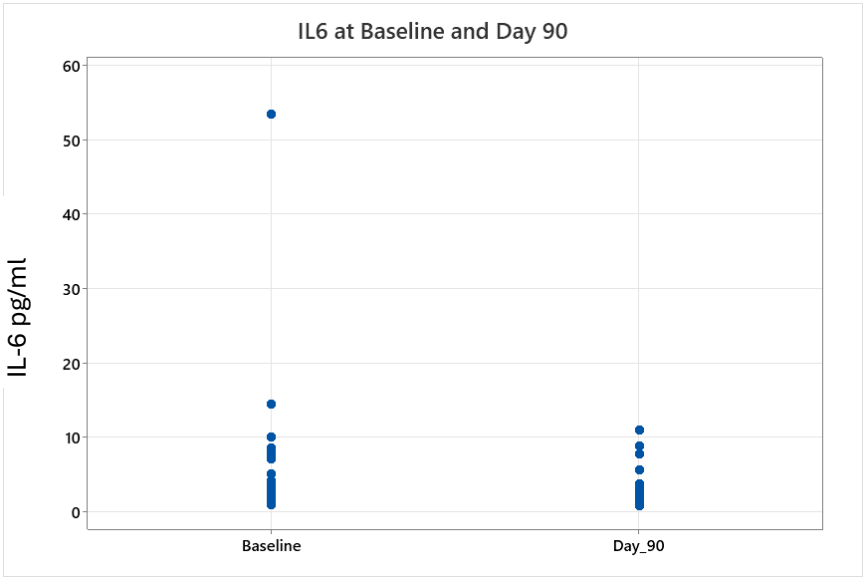


Figure 2.5: IL-6 at Baseline and Day 90
IL-6 measured by Luminex in saliva, at baseline and following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)in patients with periodontitis

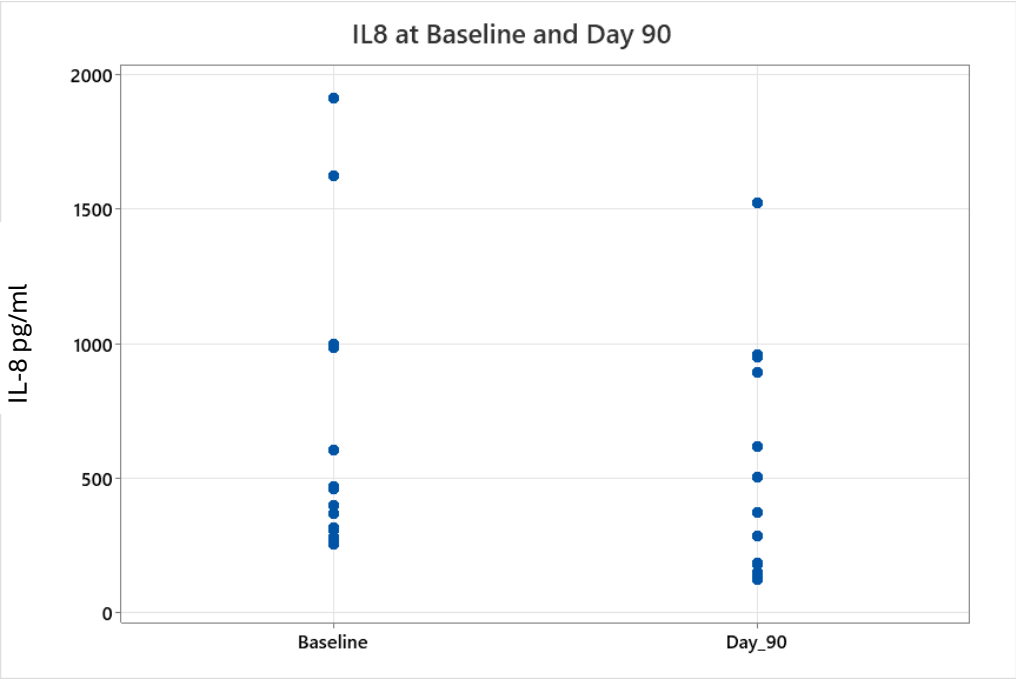


Figure 2.6: IL-8 at Baseline and Day 90
IL-8, measured by Luminex in saliva, at baseline and following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)in patients with periodontitis

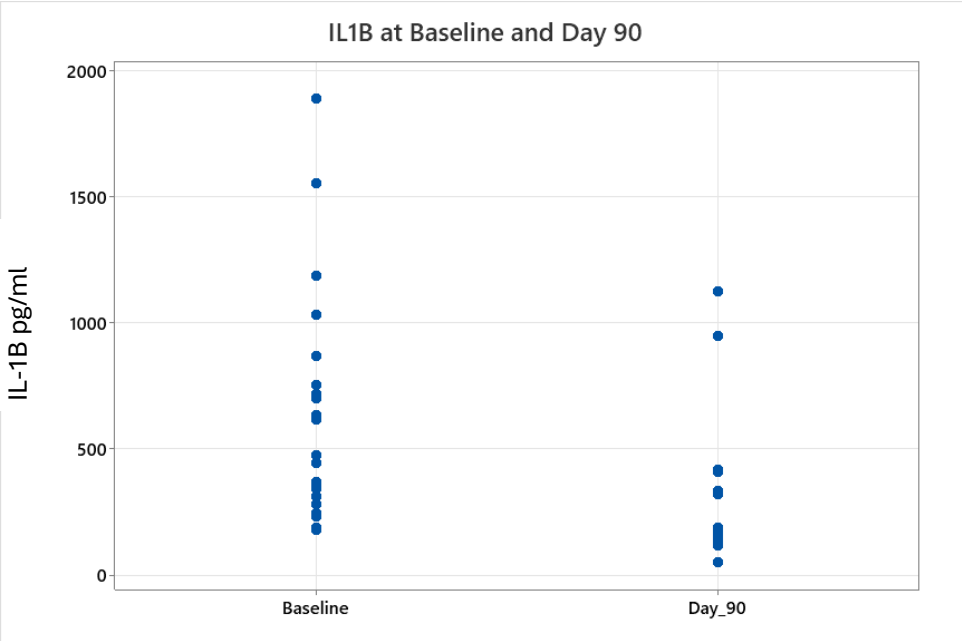


Figure 2.7: IL1 β at Baseline and Day 90
IL-1B, measured by Luminex in saliva, at baseline and following non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)in patients with periodontitis

2.2.3 Evaluation of relationship between OPG, RANKL and clinical inflammation

To investigate research question 1A:

"In patients who are systemically healthy with periodontitis, or in healthy volunteers with gingivitis or periodontal health: do salivary concentrations of OPG, RANKL relate to the clinical state of the periodontal tissues (health, gingivitis and periodontitis?" associations between these mediators and clinical findings were evaluated.

There were insufficient detectable data points from RANKL to perform any analyses.

Relationship of OPG concentrations to clinical status

Salivary OPG concentration with respect to clinical status is shown in Figure 2.8A which shows salivary OPG in health, gingivitis and periodontitis (at baseline). Figure 2.8B shows salivary OPG in health, gingivitis and periodontitis following treatment (Day90). Both before and after non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy), a small number of periodontitis patients showed elevated OPG in saliva. These data are represented in table 2.3. A Kruskal Wallis test indicated that there were no differences in OPG in any of the groups. The median levels of OPG at baseline were higher in the periodontitis group than the healthy or gingivitis groups (63.9pg/ml vs 19.5pg/ml vs 21.2 pg/ml) and there was notable variation in OPG levels at baseline in the periodontitis group. However, there were no statistically significant differences between the groups.

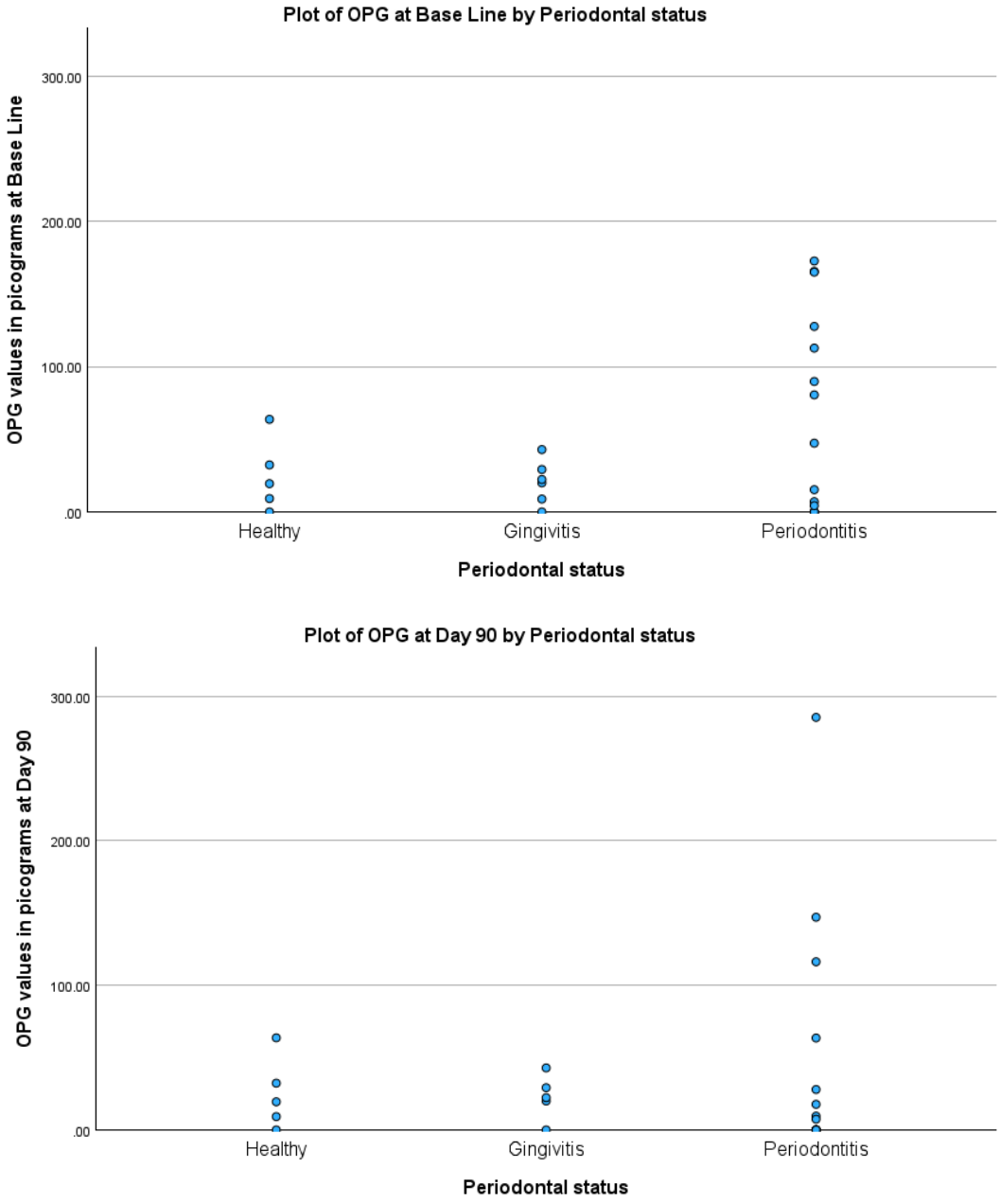


Figure 2.8A and B
OPG was measured by Luminex in saliva, obtained from volunteers who had either healthy gingival tissues or gingivitis, or from patients with (A) untreated and (B) treated periodontitis. (non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy))

Table 2.3. Summary statistics and Kruskal Wallis test for salivary levels of OPG at baseline and at Day 90 according to Periodontitis status (healthy, gingivitis, periodontitis).

	Healthy (baseline only)			Gingivitis (Baseline only)			Periodontitis			Kruskal Wallis p-value
n	5			5			14			
	Median	(Q1,Q3)	(Min,Max)	Median	(Q1,Q3)	(Min,Max)	Median	(Q1,Q3)	(Min,Max)	
OPG at Baseline	19.5	(4.6,48.3)	(0,63.7)	21.2	(6.7,36.1)	(0,42.9)	63.9	(3.2,137)	(0,172.6)	0.5
OPG at Day 90 *	19.5	(4.6,48.3)	(0,63.7)	21.2	(6.7,36.1)	(0,42.9)	8.5	(0,76.8)	(0,285.4)	0.8

* The Values for health and gingivitis at baseline are replicated to compare the effect of periodontal treatment (non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)) in periodontitis patients on salivary levels of OPG

2.2.4 Evaluation of relationship between cytokines IL-6, IL-8 and TNF- α and clinical inflammation

To investigate research question 1b: “In patients with periodontitis, do salivary cytokines (IL6, IL- 1ß,IL-8, TNF- α) associate with the periodontal inflamed surface area (PISA) at 0 and 90 days (before and after periodontal treatment)? Associations between PISA at baseline and at day 90 after treatment, and the cytokine concentrations were evaluated.

The data comparing salivary cytokines with PISA before periodontal treatment are shown in Figure 2.9 a and c. Prior to periodontal treatment, there were no correlations between salivary cytokine concentration and PISA, as assessed by Spearman’s Rho test (Table 2.4).

Similarly, following periodontal treatment there were no significant associations between PISA and any of the salivary cytokines and OPG evaluated (Table 2.5 and figure 2.9 b,c).

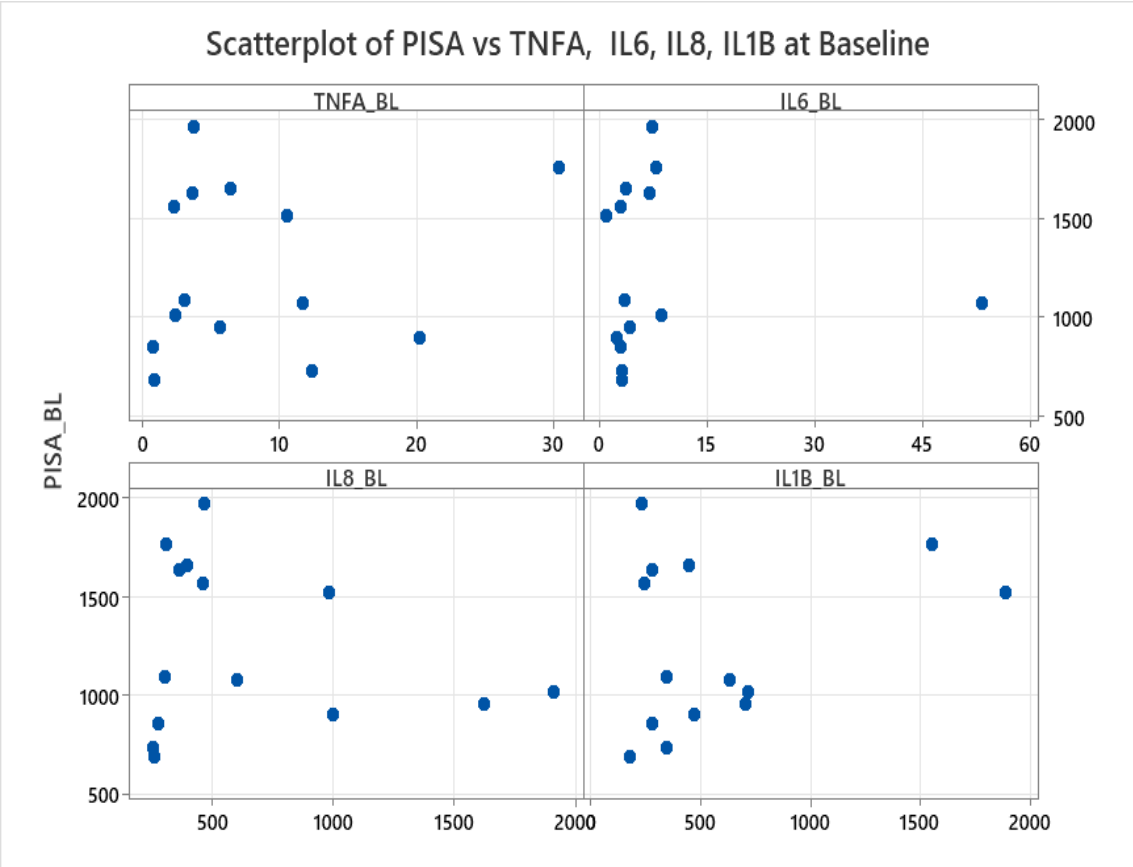


Figure 2.9 a. Cytokines in saliva was measured by Luminex in saliva from patients with (A – upper panel) untreated and (B - lower panel) treated periodontitis (non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)). Data are represented as scatterplots of PISA vs TNF- α, IL6, IL8, IL1B at baseline

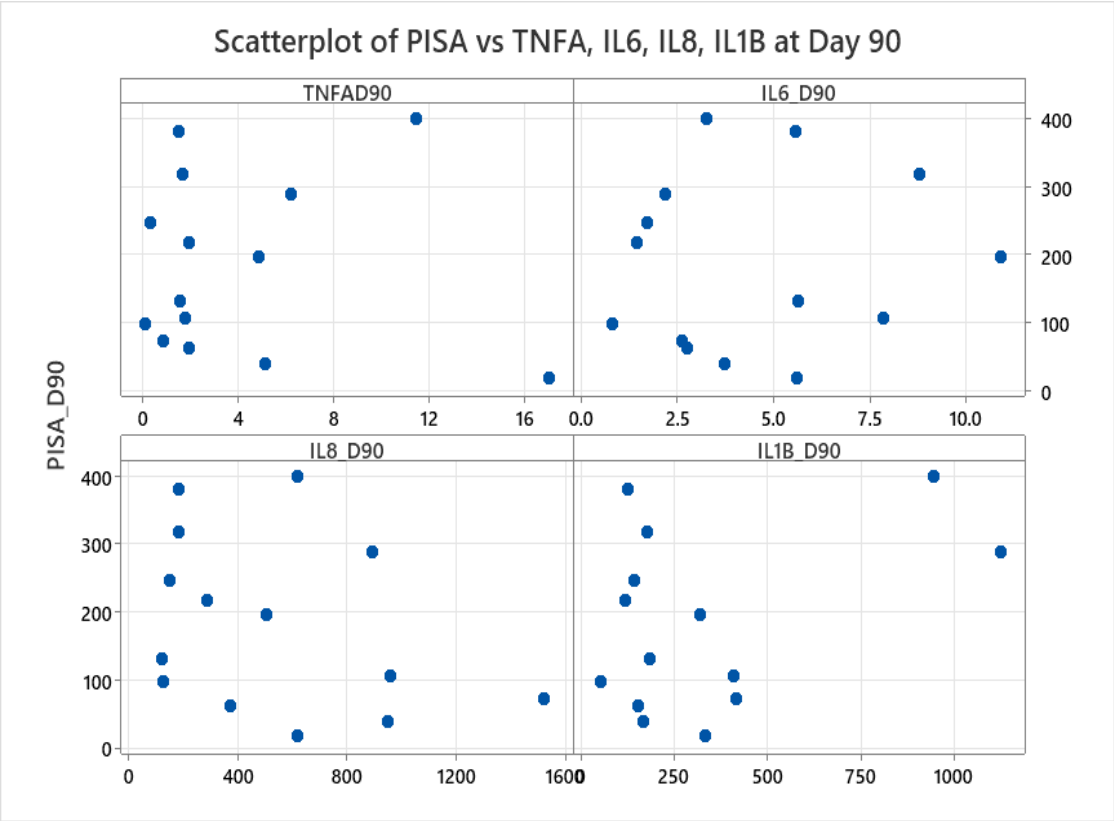


Figure 2.9 b. Cytokines in saliva were measured by Luminex in saliva from patients with (A – upper panel) untreated and (B - lower panel) treated periodontitis (non-surgical periodontal treatment (encompassing step 1 and step 2 of therapy)). Data are represented as scatterplots of PISA (mm^2) vs TNF- α, IL6, IL8, IL1B at day 90.

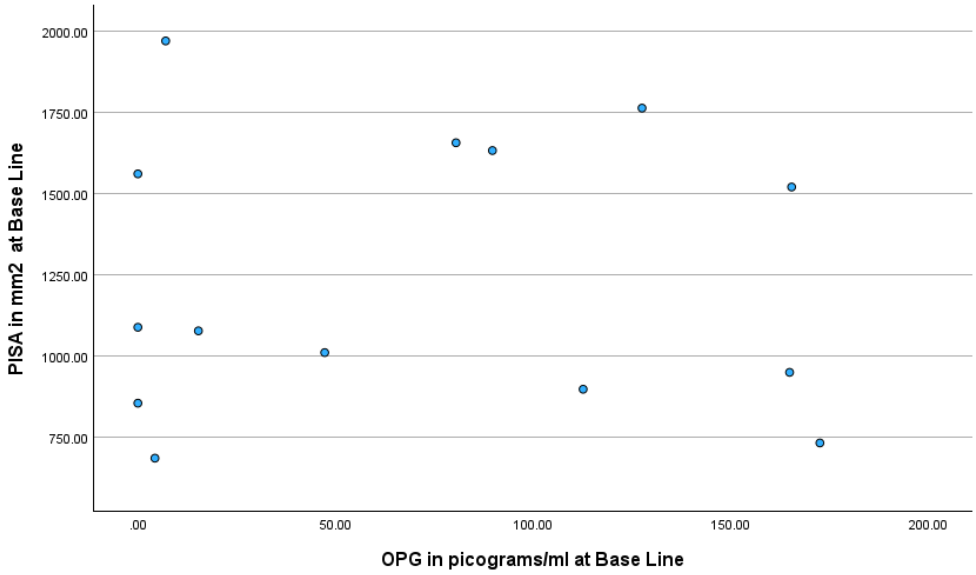


Figure 2.9c Plot of PISA in mm^2 v.s. salivary levels of OPG in picograms/ml at Base line in periodontitis patients

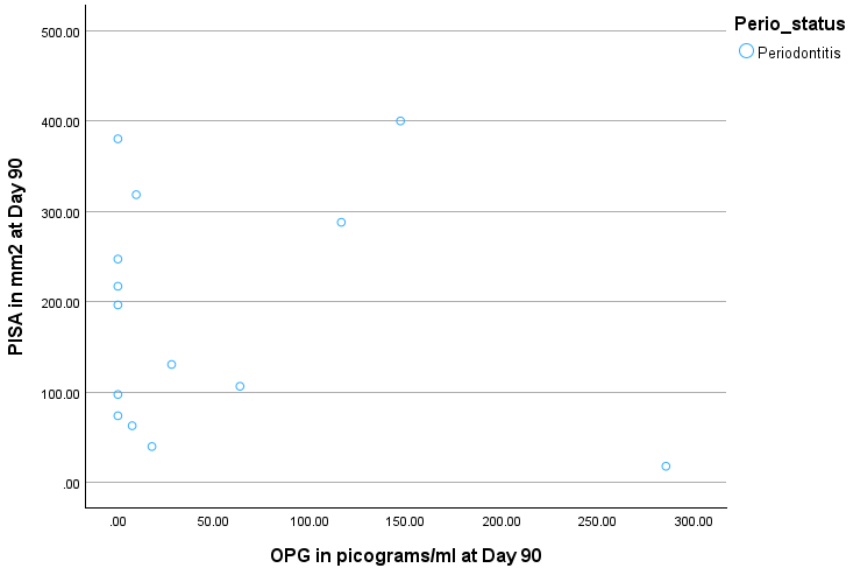


Figure 2.9d Plot of PISA in mm^2 v.s. salivary levels of OPG pg/ml at Day 90 (post treatment) in periodontitis patients

Table 2.4. Spearman's Rho correlations between PISA and TNF- α, IL6, IL8, IL1 β at baseline.

Sample 1	Sample 2	N	Correlation	95% CI for ρ	P-Value
TNF- α _BL	PISA_BL	14	0.213	(-0.364, 0.672)	0.464
IL6_BL	PISA_BL	14	0.389	(-0.200, 0.771)	0.169
IL8_BL	PISA_BL	14	0.196	(-0.379, 0.661)	0.503
IL1 β _BL	PISA_BL	14	0.134	(-0.429, 0.622)	0.648

Table 2.5. Spearman's Rho correlations between PISA and TNF- α, IL6, IL8, IL1 β at Day 90.

Sample 1	Sample 2	N	Correlation	95% CI for ρ	P-Value
TNF- α D90	PISA_D90	14	-0.090	(-0.593, 0.464)	0.759
IL6_D90	PISA_D90	14	0.024	(-0.513, 0.548)	0.935
IL8_D90	PISA_D90	14	-0.323	(-0.736, 0.265)	0.260
IL1 β _D90	PISA_D90	14	0.059	(-0.487, 0.572)	0.840

In summary, the results from this chapter show that OPG is detectable in some salivary samples from both healthy, gingivitis and periodontitis patients. OPG concentrations in saliva did not show any association with disease state. RANKL was undetectable in any of the samples analysed.

Chapter 3. An observational clinical study of oral inflammation in patients who start taking denosumab for osteoporosis treatment.

Hypothesis:
Systemic inhibition of RANKL (during treatment for osteoporosis) will impact on cytokine levels in saliva and GCF and impact on gingival inflammation.

This chapter sought to evaluate Research Question 2:

> Do patients who start treatment with denosumab for osteoporosis show evidence of changes in the oral cavity associated with altered periodontal health?

3.1 Clinical study

The proposed study planned for this project received REC approval from the West Midlands - Coventry & Warwickshire Research Ethics Committee: 20/WM/010; IRAS project ID: 245083; and NHSGG&C management approval GN17OD423. The study protocol, patient information leaflet and consent form are shown in appendix 1A and 1B respectively.

This study was approved in early 2020, and the study start considerably delayed due to COVID-19 restrictions on clinical research. During the period of the delay, the prescribing regulations and recommendations for Denosumab changed. This was following the identification of rapid rebound of osteoclast activity following discontinuation of denosumab, which resulted in vertebral fractures in 10% of patients. Given the notable issues with stopping denosumab, and the need for a long-term plan for patients to continue anti-resorptive therapy after halting denosumab, the number of patients who were starting denosumab (ie the target population of this study) rapidly diminished to near zero.

A single patient was recruited to the study, who completed the study. The patient was diagnosed as having gingivitis. The analysis of their salivary RANKL and OPG was carried out as for previously described samples and the results are shown in Table 3.1. No conclusions could be drawn from the single sample. As the study subsequently was unable to recruit any other patients who were starting denosumab, the study was halted. Although the study did not go ahead as planned there were considerable learnings from the study approval processes. Moreover, this work highlighted the challenges of studying populations with two conditions (periodontitis and osteoporosis), and the challenges of studying complex biologic medications.

Table 3.1: Salivary RANKL and OPG levels in picograms/ml at Baseline and Day 90 in Clinical observational study cohort

POD								
n	RANKL_BL		RANKL_D90		OPG_BL		OPG_D90	
1	0*	0*	0*	0*	17.747	0*	33.3	29.889

(Values from luminex are retained in duplicate and a 0* indicates levels below Limit of detection.)

Chapter 4. General Discussion

4.1 Measuring Cytokines in Periodontitis: Benefits and Limitations

This thesis looked at concentrations of cytokines, IL-1β, TNF-α, IL-6, RANKL and OPG in samples from the oral cavity. There were notable challenges with detecting these mediators in salvia – in particular RANKL and OPG. The assay employed was among the most sensitive on the market. Future technologies may permit more sensitive assays. Measuring these mediators in tissues may be more relevant and allow detection of higher concentrations. In particular, the apparent absence of RANKL in the clinical samples (or at least below level of detection) was surprising as previous studies have documented elevated RANKL in clinical samples (Buduneli, 2008). There is the possibility that as the samples from patients with periodontitis had been stored for several months that the lack of detectable cytokine may be a result of prolonged storage. Nonetheless, measurement of cytokines in samples from patients with periodontitis as a means to inform potential host modulation strategies is a valid approach. Several cytokines are known to be central to the pathogenesis of periodontitis, reflecting the host's inflammatory and osteoimmune response (Preshaw, 2011). Quantifying these mediators in gingival crevicular fluid (GCF), saliva, or serum provides insight into disease activity and could be used to evaluate the biological impact of host modulation therapy (Figure 5.1) (Rakic, 2025).

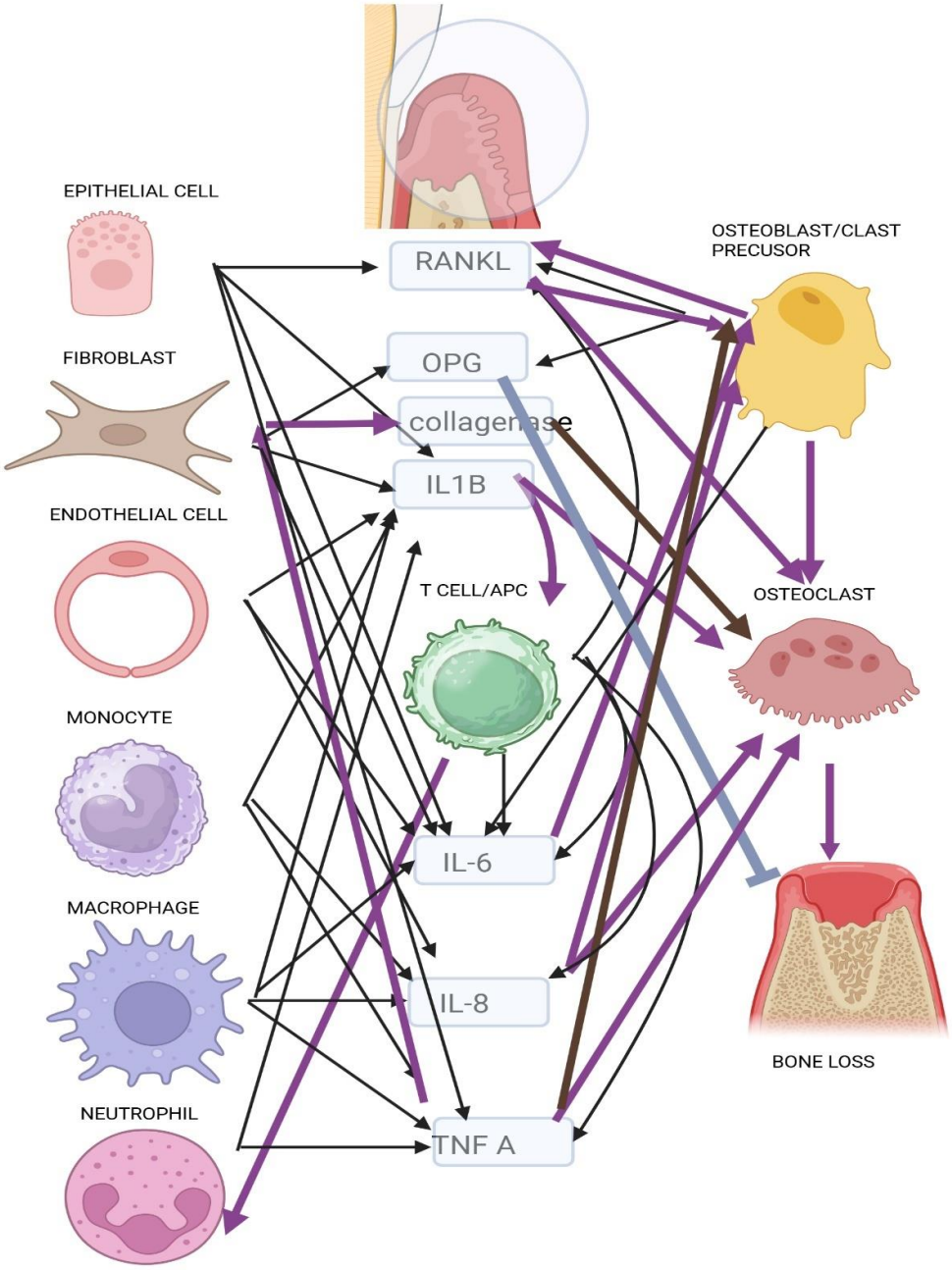


Figure 4.1: Schematic representation of the complexity of inflammatory process in the gingiva of a periodontitis patient. The mediators named in the centre of the diagram are potential targets, and therapeutics targeting these mediators are already in use in other diseases.
Key → secretes/produces → Activates ⊣ Inhibits (made with Biorender)

Cytokine profiling offers a window into the local inflammatory milieu and the patient's systemic inflammatory status. Elevated IL-1ß, TNF-α, and IL-6 levels correlate strongly with attachment loss, bone resorption, and disease progression (Graves et al., 2008). Monitoring cytokine dynamics before and after therapy can identify responders to HMT, distinguishing inflammation resolution from mere microbial suppression. Non-invasive GCF and salivary assays are repeatable and well-tolerated, providing a practical tool for longitudinal follow-up. Furthermore, multiplex platforms allow simultaneous measurement of numerous mediators, facilitating complex immune profiling that aligns with the principles of precision medicine.

Despite scientific promise, cytokine measurement has yet to transition from research to routine clinical use. Variability in sample collection, diurnal fluctuation, and influences from systemic conditions such as diabetes or smoking reduce reproducibility (Engebretson & Lamster, 2013). There is no consensus on threshold values for disease activity, and cytokines rarely act independently; their interpretation requires integration with microbial, genetic, and clinical data. Moreover, commercial assays differ in analytical sensitivity, complicating cross-study comparison.

Combining cytokine data with machine-learning algorithms and multi-omic integration could enable predictive modelling of disease progression or therapeutic response (Teles & Teles, 2021). Real-time, chairside biosensors and salivary diagnostics are emerging, potentially enabling personalised monitoring of host response and timely adjustment of HMT regimens.

4.2 Studying the Effects of Biologics Used for Other Diseases on Periodontal Health

Systemic biologic therapies originally developed for autoimmune or inflammatory conditions—such as rheumatoid arthritis —offer a natural experiment for understanding how targeted cytokine inhibition influences periodontal inflammation (Petit et al 2024). Agents such as TNF-α inhibitors (infliximab,

etanercept), IL-1 receptor antagonists (anakinra), IL-6 receptor blockers (tocilizumab), and IL-17 inhibitors (secukinumab) are widely used in medicine and provide mechanistic insights relevant to host modulation in periodontitis (Siebert, 2015). This study sought to evaluate the effect of denosumab, by investigating patients prescribed Prolia for osteoporosis. The clinical trial in this study highlights several complexities and challenges with this approach. In particular, the patient population needs to be recruited from a distant clinic, relying on non-dental teams and clinicians. This makes recruitment especially challenging given the inevitable pressures on all outpatient clinics. The dental team visiting the osteoporosis clinics and met the osteoporosis team who were extremely helpful and enthusiastic. Nonetheless, inevitably the clinics are busy and studies are easily forgotten. In addition, there is seldom a dedicated 'one disease/one drug' clinic – so the target group for recruitment appear sporadically interspersed with other complex patients.

The change in direction for indications and use of denosumab was the 'final blow' to recruitment to this study. This highlights both a strength and weakness of this type of 'repurposing' approach – the profile and effects of the drug in question and well known and information will continue to be gather. However, this may throw up favourable as well as unfavourable changes in direction. Therefore, pharmacovigilance data and established safety profiles provide a foundation for repurposing efforts without duplicating early-phase toxicology studies -but clearly additional unwanted side effects can emerge over time!

The observational studies indicate that RA patients receiving TNF-α inhibitors exhibit reduced periodontal inflammation and lower levels of inflammatory mediators compared with untreated controls (Ortiz et al., 2019) suggest systemic biologic therapy may confer secondary periodontal benefits. These findings strengthen the concept of shared inflammatory pathways and justify the exploration of biologics as potential periodontal modulators. Moreover,

However, translating systemic biologic use into dentistry is extremely complex. Biologics are expensive, mostly currently require parenteral delivery (and this method of delivery is the basis of all the current data), and carry risks of

immunosuppression, infection, and malignancy. Periodontitis typically affects otherwise healthy adults, making systemic immunomodulation ethically and economically challenging outside of specific comorbid contexts. Moreover, disease heterogeneity in periodontitis complicates extrapolation—cytokine dominance patterns differ between tissues.

Arguable, the next phase of translational research will likely involve local delivery of biologics—such as IL-1 or RANKL inhibitors—within the periodontal pocket, combining the potency of biologic modulation with the safety of site-specific therapy. Rigorous mechanistic studies are needed to delineate which biologic pathways are most relevant to periodontal inflammation, possibly identifying subsets of patients who could benefit from adjuvant biologic therapy within a personalised-care model.

4.3 Drug Repurposing: Opportunities and Challenges

As noted above, this approach of evaluating drugs already in use that could also have periodontal benefits is a type of drug repurposing: using approved drugs for new therapeutic applications. This has the advantages of leveraging existing safety, manufacturing, and pharmacokinetic data. Several systemic and locally applied agents originally developed for cardiovascular, metabolic, or inflammatory diseases have shown periodontal benefits.

Repurposed drugs such as statins, tetracyclines, NSAIDs, and bisphosphonates have demonstrated anti-inflammatory, antioxidant, and bone-anabolic effects in periodontal tissues (Golub et al., 1999; Pradeep & Thorat, 2015). Subantimicrobial-dose doxycycline (SDD) modulates matrix metalloproteinases without exerting antimicrobial selection pressure and remains the only FDA-approved HMT for periodontitis (Khattri, 2020).

More recently, complement inhibitors such as AMY-101 (a C3 compstatin analogue) have been repurposed from systemic inflammatory indications and tested locally in periodontal disease, via a mouthwash, showing substantial

reductions in gingival inflammation in a Phase IIa clinical trial (Hasturk et al., 2021).

Repurposing also encourages cross-disciplinary learning: discoveries in rheumatology or oncology can rapidly inform periodontal innovation, fostering a translational continuum between medicine and dentistry.

Repurposed drugs often lack tissue specificity. Systemic exposure may produce off-target effects such as osteonecrosis of the jaw (ONJ) seen with antiresorptives. Dosing for oral tissue penetration differs from systemic pharmacology, requiring reformulation for local application. Additionally, commercial incentives are challenging once patents expire, limiting industry-funded development. Regulatory frameworks for new dental indications or currently available drugs are as yet largely unexplored.

Progress will depend on mechanistic justification, biocompatible delivery systems, and adaptive clinical-trial designs capable of validating efficacy with smaller, stratified cohorts. Partnerships between academia and small biotech companies may accelerate innovation in this area.(Hasturk, 2021)

4.4 Local Drug Delivery for Periodontitis and Biologic Agents: State of the Art and Future Opportunities

As noted above, local drug delivery (LDD) may prove transformative if biologics are to be incorporated into periodontal therapy aims. Local drug delivery aims to achieve high local drug concentrations while minimising systemic side effects. The subgingival pocket offers a contained, accessible site for controlled release of antimicrobials, anti-inflammatory agents, and, increasingly, biologics.

Commercially approved systems include the chlorhexidine chip (PerioChip), 10% doxycycline gel (Atridox), and minocycline microspheres (Arestin®)—each providing sustained release of active agents within the pocket (Jeffcoat et al., 1998; Williams et al., 2002). These adjuncts to SRP yield modest but statistically significant improvements in probing depth and clinical attachment. Experimental

approaches using statin gels or microspheres demonstrate potential bone-regenerative effects (Rao et al., 2013).

Conventional carriers (chips, gels, microspheres) often exhibit limited retention and uneven distribution due to pocket fluid dynamics – in particular if seeking local delivery of proteins such as biologics. Manufacturing biologic-loaded devices poses challenges in stability, sterility, and cost. Regulatory pathways are further complication because these products straddle drug-device classifications.

Advances in biomaterials and nanotechnology might to overcome many of these hurdles. Bioresponsive hydrogels, pH- or enzyme-triggered release systems, and nanoparticle carriers are being designed for sustained, site-responsive drug release (Patel et al., 2021). Hybrid regenerative scaffolds that combine host modulators, antimicrobials, and growth factors may both control inflammation and promote bone regeneration. Integration with biomarker-guided diagnostics could enable personalised "on-demand" therapy targeted to sites of active inflammation.

In the longer term, smart LDD systems could interface with digital diagnostics, allowing real-time feedback-controlled drug release based on local cytokine levels—a true manifestation of precision periodontics.(Jeffcoat, 1998)(Williams, 2001)(Rao, 2013)(Patel, 2021)(Hasturk, 2021).

4.5 Integration of Host Modulation Therapy into Clinical Practice: Future Opportunities

Integration of HMT into mainstream periodontal practice could be facilitated and optimised by personalised approaches that match therapeutic agents to each patient's biological profile. Periodontitis encompasses diverse host–microbe interactions driven by genetic, epigenetic, and environmental factors. Stratifying patients by inflammatory endotypes—for example, IL-1 β hyper-responders or high MMP producers—may identify those who benefit most from specific host modulators (Tonetti & Jepsen, 2022). Biomarkers may guide adjustment of therapy. Monitoring

GCF or salivary levels of MMP-8, IL-1β, or RANKL/OPG could indicate when to initiate, intensify, or discontinue HMT. Although not yet validated for routine practice, commercial point-of-care kits are emerging.

4.6 Conclusion

Periodontal disease has been reframed as a disorder of immune dysregulation rather than solely microbial infection. Measuring cytokines and other biomarkers offers a pathway toward personalised precision care. Drug development and/or repurposing and biologic adaptation create opportunities for step changes in periodontal therapy. Local delivery systems might overcome some of the potential challenges of host modulation, offering targeted efficacy with minimal systemic risk. Integrating these advances into daily practice will require interdisciplinary cooperation, cost-effective diagnostics, and long-term safety data.

The emergence of the complement inhibitors and resolvins in clinical trials shows there is potential. It seems likely that future of periodontal therapy lies in combining mechanical debridement with tailored host modulation. As technologies mature, HMT could evolve from an adjunctive concept into a cornerstone of regenerative and preventive periodontal medicine, bridging oral and systemic health. Future work, for example, integrating larger scale dental assessments for patients starting biologics would benefit patient care from a dental health standpoint – and inform this field substantially.

Acknowledgements

The cytokine analysis of clinical samples was funded by NHSGG&C Endowment funding. The Luminex analysis was carried out with the assistance of Robert Reilly and School of Infection and Immunity Flow Cytometry Facility at the University of Glasgow. Artificial intelligence tools (University of Glasgow Microsoft Co-pilot) were employed to assist with English language and grammar correction during thesis preparation.

Appendix 1A

Clinical Trial Protocol

Periodontal Health in patients on Denosumab (PoD)

Running title: **Periodontal Health in patients on Denosumab (PoD)**

Protocol version: **Version 1.1**
Date: **15th May 2020**
REC Reference Number:
ISRCTN/Clinical trial.gov:
Sponsor's Protocol Number:

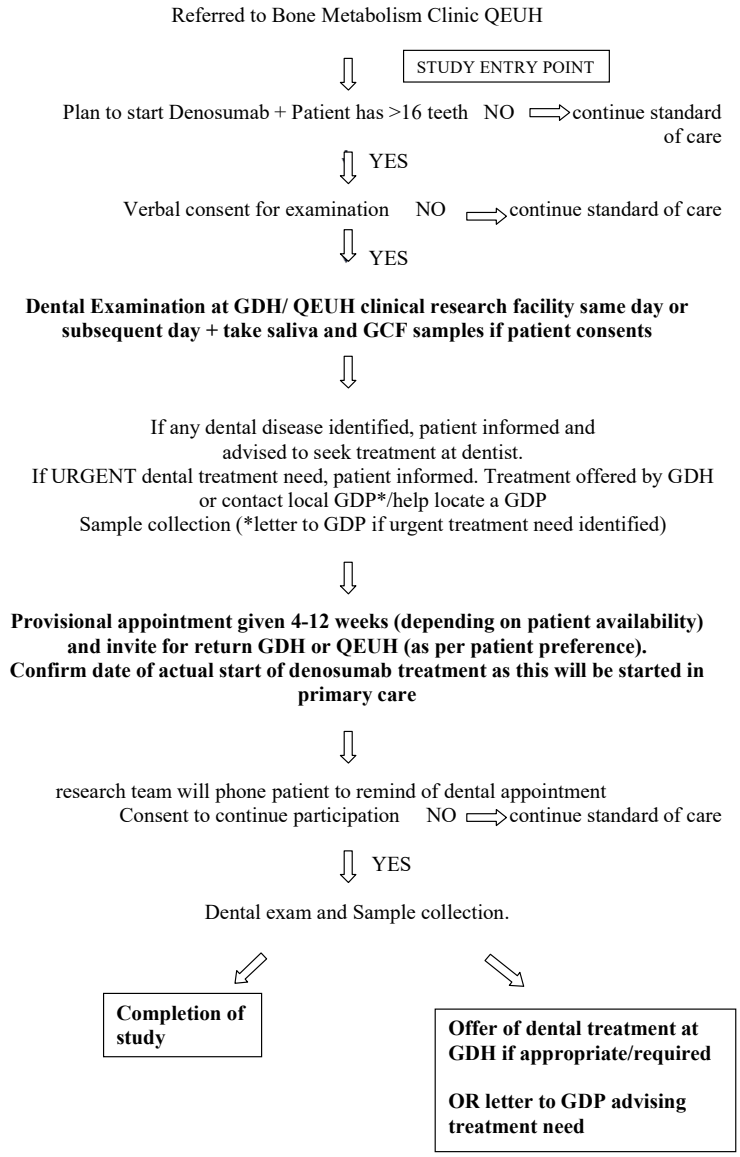
Sponsor: NHSGGCR&D
Funder: NHSGGC Endowment

ABBREVIATIONS

CI	Chief Investigator
CRF	Case Report Form
BOP	Bleeding on Probing
LOA	Loss of Attachment
PPD	Probing Pocket Depths
GCF	Gingival Crevicular Fluid
GDCRF	Glasgow Dental Clinical Research Facility
QEUH	Queen Elizabeth University Hospital
PD	Periodontal Disease
PIL	Participant Information Leaflet
R&D	Research and Development
REC	Research Ethics Committee
SD	Standard Deviation
BMC	Bone Metabolism Clinic
GDCRF	Glasgow Dental Clinical Research Facility
QEUH CRF	Queen Elizabeth University Hospital Clinical Research Facility

Title of Study:	The effects of Denosumab on Periodontal Health
Study Centre:	Glasgow Dental Hospital and School & QEUH CRF
Study Duration:	30 months
Primary Objective:	To identify effects of Denosumab/anti-resorptive bone medication on inflammation in the mouth.
Secondary Objective:	To identify effects of Denosumab/anti-resorptive bone medication on periodontal health e.g. LOA, PPD and % bleeding sites, and effects on immune response in the mouth, and the oral microbiome.
Primary Endpoint:	Change in inflammation after initiation of anti-resorptive treatment assessed by - clinical (bleeding on probing) and biological measures (salivary and GCF cytokines).
Rationale:	We propose that targeting RANKL could be a useful adjunct to standard treatment for advanced gum disease, and thus improve oral health and related quality of life. We seek to first evaluate changes in oral health following routine use of this medication for osteoporosis. We plan to seek a larger follow-on trial designed to evaluate the impact of systemic administration of anti-resorptive agents on oral health.
Methodology:	Sample collection study
Sample Size:	20 patients
Screening:	Patients referred to the Bone Metabolism Clinic in QEUH and commencing Denosumab/antiresorptive therapy
Registration/ Randomisation:	Patients and their samples will be assigned a study code. There is no randomisation.
Main Inclusion Criteria:	• Written informed consent • Male or female aged 18 years and over • Have at least 16 natural teeth • Due to start Denosumab or other antiresorptive medication for the treatment of osteoporosis.
Main Exclusion Criteria:	• Known or suspected high risk of tuberculosis, hepatitis B or HIV infections • Require interpreter/non English language written material to understand and provide, or any other reason for being unable to provide written, informed consent • History of bleeding diathesis • Need for antibiotic cover for dental examination
Statistical Analysis:	Different types of data will be generated from the study, e.g.

	Clinical parameters, gene and protein expression at start of medication and up to3 months after start of medication. Confounding factors e.g. smoking will be recorded. Data will be analysed appropriately under the guidance of the study statistician. Data will be evaluated for normal distribution. For example, paired data may be analysed by Wilcoxon tests. Independent data may be analysed by T test or Mann-Whitney U-test. Correlations between parameters may be investigated by Spearman rho.



STUDY FLOW CHART

SCHEDULE OF ASSESSMENTS

Study Procedure	Screening visit at BMC	(Visit I at GDCRF or QEUHCRF)*	Visit 2at GDCRF or QEUHCRF)
Study information given	●		
Review inclusion/exclusion criteria	●	●	●
Obtain indication if patient will consider study	●		
Written consent obtained	●		
Review consent		●	●
Dental examination, oral hygiene advice and sample collection (saliva, GCF, plaque)		●	●
Complete CRF	●	●	●
Patient informed of any dental disease present and appropriate advice given/options explained		●	●

***Note - Visit I can be same day as screening visit (at QEUH), or can be a later date of patient's preference, at either QEUH OR GDH depending on patient preference.**

1 INTRODUCTION

1.1 Background

Severe gum disease (periodontitis) affects 10% of the population. Periodontitis causes tooth loss, compromising oral health and quality of life. The tooth loss results from destruction of the bone supporting the teeth. The bone around the teeth is destroyed by 'friendly fire' from the immune system. Current gum treatment focuses on the removal of a biofilm from the teeth. This treatment is resource intensive, often only partially successful and disease recurrence is common. The destruction of bone surrounding teeth in advanced gum disease is caused by an aggressive immune response to the biofilm of microbes. There is an unmet need to find new ways to keep teeth healthier throughout life. Targeting the immune response could be an effective aid to standard gum treatment.

We are interested in a drug called Denosumab, which is used to reduce fracture risk in patients with osteoporosis. Denosumab stops bone loss so might also help patients with periodontitis. However, long term use of Denosumab is associated with a small increase in risk of osteonecrosis of the jaw. We seek to examine, in detail, the mouths of patients who start taking Denosumab. This may help patients with osteoporosis and patients with periodontitis.

1.2 Rationale

We have identified different immune components that share a common pathway to bone destruction. We think that this pathway is a useful target for the treatment of gum disease. We have published findings from in vivo and in vitro of model systems. The research shows that immune cells and cytokines control bone loss. The cytokine called 'receptor activator nuclear factor B ligand' or RANKL is crucial for bone loss in experimental systems of periodontal disease. RANKL can be inhibited by another protein called osteoprotegerin (OPG). In a rodent model of gum disease, provision of OPG to inhibit RANKL eliminated periodontal bone destruction.
RANKL is currently targeted to prevent fractures associated with osteoporosis, via a drug called Denosumab. This drug binds to and stops RANKL. We propose that targeting RANKL could be a useful adjunct to standard advanced gum disease treatment. There is currently no data documenting the changes in the oral health of patients who start taking Denosumab.

1.3 Prior experience of periodontal research

The Chief Investigator (CI) is a Professor of Periodontology and Immunology and Specialist Periodontist, treating patients with advanced forms of periodontal disease. The CI has developed a track record in periodontal research, with research funding in the region of £1.3 million and a number of peer reviewed publications as detailed on attached CV. The CI works in close collaboration with world-renowned immunologists and microbiologists and several Consultant Periodontist colleagues at the Glasgow Dental Hospital.

1.4 Study hypothesis

Antiresorptive therapy can modify inflammation and bone destruction in the oral cavity

2 STUDY OBJECTIVES

This is a sample collection study from patients commencing anti-resorptive medication to reduce bone turnover e.g. Denosumab. This will allow evaluation of the inflammation in the mouth before commencing this medication and 4-12 weeks after commencement. The aim is to identify whether Denosumab bone medication affects inflammation in the mouth.

Primary Endpoint

Change in percentage sites in the mouth with BOP following initiation of anti-resorptive treatment (a measure of inflammation in the mouth.)

Secondary Endpoint

a) Biological changes following initiation of anti-resorptive treatment,

- change in cytokines in GCF and saliva
- change in PPD, attachment levels, plaque scores
- changes in antibodies against bacteria associated with gum inflammation in saliva
- changes in composition of microbial biofilm in the mouth

b) Comparison of changes in patients who start taking Denosumab with patients who take bisphosphonates (an anti-resporptive with a different mechanism of action).

3 STUDY DESIGN

This sample collection study will be performed according to the Research Governance Framework for Health and Community Care (Second edition, February 2006)

3.1 Study Population

We will recruit patients attending the Bone Metabolism Clinics in Glasgow. Looking at from population studies, (age group 18-75 years) we anticipate around 80% of patients will have at least 16 teeth. We will investigate two groups:

- 10 Patients starting Denosumab for osteoporosis
- 10 Patients starting Bisphosphonates for osteoporosis

The inclusion criteria **are**

- male or female aged 18 and over
- Written informed consent
- Must have at least sixteen natural teeth remaining
- Patients starting Denosumab for osteoporosis
- OR Patients starting Bisphosphonates for osteoporosis

The exclusion criteria **are**

- Known of suspected high risk for tuberculosis, hepatitis B or HIV infection
- Require interpreter/non-English language written material to understand and provide or any other reason for being unable to provide written, informed consent
- History of bleeding diathesis
- Patients requiring antibiotic cover for dental examination

There will be no randomisation of patients

3.2 Identification of participants and consent

At the bone metabolism clinic, patients will be initially identified and approached by a member of the dental research time (the CI or research dental nurse/ hygienist/ specialist or core trainee). Patients will be screened at the time to establish if they are eligible. The inclusion/exclusion criteria will be evident from the information taken as part of a normal clinic (history, exam, treatment plan). Eligible patients will be initially informed of the existence of the study. After the patient is made aware of the study, they will be invited to ask questions. If willing to participate, the dental research team member will obtain consent. At each stage, it will be made clear to the patient that their participation is voluntary. They can leave the study at any time without their care being affected.
This initial contact will be to provide the patient with the PIL and establish whether they would like to take part, with the possibility of dental exam and

collection of saliva and GCF samples the same day if more convenient for the patient. Alternatively, the patient will be provided with the chance to take away the information and consider their participation in the study. They can then make an appointment at their convenience for dental exam and sample collection.

3.3 Withdrawal of subjects

Patients will be withdrawn from the study if they are no longer eligible (e.g. due to a change in general health) or if the patient withdraws their consent to participate, or if for any other reason the CI or patient's consultant or other clinician involved in their care deems it detrimental to the patient to continue to participate. Withdrawal will be immediate. The CI will be informed and will confirm whether the patient wishes any previously obtained samples and associated data to be withdrawn from the study. The reason for withdrawal and the subsequent action taken will be documented on a case-by-case basis as an addition to the case report form.

4 TRIAL PROCEDURES

4.1 Study schedule

Patients will attend the Bone Metabolism Clinic at the QEUH. Patients will require two appointments for dental examinations, data collection and sample collection appointments. The first can either be on the same day as their Bone Metabolism Clinic appointment, or at a subsequent appointment at either QEUH or Glasgow Dental Hospital – location and time will be according to patient preference. The second appointment will be at either location, again, depending on patient preference regarding site and time of appointment.

Screening Visit

Patients are appointed to a Bone Metabolism Clinic at which a clinical history and clinical examination are completed by the consultant in charge, or his/her representative (specialist or core trainee). Patients at this appointment will be identified who meet the inclusion criteria noted above. Appropriately identified patients will then be approached by the CI or her representative (clinical research nurse/hygienist/specialist or core trainee). Patients will be informed of the existence of the study and if they show interest, a patient information leaflet will be provided for them to read regarding the details of the study. If the patient is still interested, the patient will have the opportunity to review the consent form. If the patient wishes to participate, a dental examination can be carried with saliva and GCF sample collection at the QEUH CRF. However the patient will also be asked if they would rather return at a different time to either the QEUH CRF or the GDH CRF. This will allow the patient more time to consider participating in the study and / or allow the patient more time for the dental examination and sample collection..

Visit 1. Patient assessment and sample collection

This is at either the same appointment as the bone metabolism appointment or an additional appointment according to patient preference. This will be at the Queen Elizabeth University Hospital or the Glasgow Dental Hospital. This will be depending on patient preference. Consent will be checked at this appointment and the dental examination and GCF, plaque and saliva samples are obtained.

Visit 2. Patient re-assessment and sample collection

This is a review visit either at the Queen Elizabeth University Hospital or the Glasgow Dental Hospital. The choice of location will be determined by patient preference. The patient consent is checked again, and dental exam completed and further GCF, plaque and saliva samples are taken. If the patient has dental treatment need at this time, the patient's dentist will be informed, and a treatment plan recommended. If treatment need is highlighted, the patient will be offered free treatment at Glasgow Dental Hospital if they wish. If the patient does not have a dentist and does not wish to avail of the hospital treatment, an offer of locating a nearby dentist will be made to the patient.

For dental exam and sample collection (saliva, plaque, GCF) prior to and after anti-resporptive medication commencement, 15-30 minutes will be required.

4.2 Study outcome measures

3.2.1 Primary outcome measure

Change in percentage sites in the mouth with BOP (a measure of gingival inflammation), following initiation of anti-resorptive treatment.

3.2.2 Secondary outcome measure

a) Biological changes following initiation of anti-resorptive treatment,

- change in cytokines in GCF and saliva
- change in PPD, attachment levels, plaque scores
- changes in antibodies against bacteria associated with gum inflammation in saliva for example anti-*Porphyromonas gingivalis* antibodies
- changes in composition of microbial biofilm in the mouth

b) Comparison of changes in patients who start taking Denosumab with patients who take bisphosphonates (an anti-resporptive with a different mechanism of action).

4.3 Laboratory tests

No NHS laboratory tests are planned,
The laboratory analysis will be carried out in University research labs using appropriate methodology (eg flow cytometry, gene expression analysis, protein detection techniques).
The laboratory analysis will evaluate:

- Change in cytokines in GCF and saliva

These will be measured by enzyme linked immunosorbant-assay (ELISA) or similar antibody based detection platform. In particular IL-1 family cytokines reflect inflammation in the mouth, and these will be evaluated first. Pending results, there will be further exploratory analysis of a panel of cytokines, chemokines, and other markers of inflammation in saliva such as matrixmetalloproteinases (MMPs).

- Changes in antibodies against bacteria associated with gum inflammation in saliva for example anti-*Porphyromonas gingivalis* antibodies, will be evaluated by anti-bacterial ELISAs looking at both IgA and IgG antibodies. Other anti-bacteria antibodies may be explored depending on results and on findings from the studies of the bacteria in the dental plaque (detailed below).
- Changes in composition of microbial biofilm in the mouth

The microbial composition of the dental plaque will be evaluated either by PCR for 16S of a panel of bacteria associated with periodontitis, or by 16S sequening to identify all the bacteria present.

5 STATISTICS AND DATA ANALYSIS

Data will be generated from coded clinical samples. Data will be managed by coding each sample and linking codes to laboratory and clinical findings. The key linking the codes to the patients will only be available to the CI and only accessed to provided further clinical information if this is required for data analysis.

There are no similar studies to this current study, looking at inflammation in the mouths of patients before and after commencing anti-resorptive medication. Therefore, this study is exploratory in nature. Once data has been obtained from the 10 patients in each group, further advice will be sought from the study statistician.

5.1 Statistical analysis

Clinical and laboratory data will be analysed by standard methods with comparison with pre medication commencement vs. post medication commencement.

Clinical data will include standard measures of gum health:

- Sites around teeth that bleed on gentle probing (%)
- Sites around the teeth that have dental plaque accumulation (%)
- Depth of periodontal pockets around teeth (mm)
- Proportion of sites that have ‘deep’ (>4mm) pockets (total number and %)
- Loss of attachment around teeth (mm)
- “Periodontally inflamed surface area, PISA” - a standard measurement calculated using the bleeding on probing and pocket depth measurements

The laboratory analysis will evaluate:

- Change in cytokines in GCF and saliva

These assays generate quantitative data

Changes in antibodies against bacteria associated with gum inflammation in saliva for example anti-*Porphyromonas gingivalis* antibodies
These assays generate quantitative data

- Changes in composition of microbial biofilm in the mouth

These assays generate quantitative and qualitative data

5.2 Primary efficacy analysis

There is no test intervention therefore no efficacy analysis.

5.3 Secondary efficacy analysis

n/a

5.4 Safety analysis

The safety data (adverse effects) – both numbers of subjects and events – will be summarised.

Adverse Event (AE) – any unfavourable and unintended sign, symptom or disease temporally associated with participation in the research project.

Serious Adverse Event (SAE) – an untoward occurrence that:

a. Results in death
b. Is life threatening
c. Requires hospitalisation or prolongation of existing hospitalisation
d. Results in persistent or significant disability or incapacity
e. Consists of a congenital anomaly or birth defect
f. Is otherwise considered medically significant by the investigator

Any SAE occurring to a research participant will be reported to the main REC where in the opinion of the Chief Investigator (CI), the event was:

- 'related' – that is, it resulted from administration of any of the research procedures, and
- 'unexpected' – that is, the type of event is not listed in the protocol as an expected occurrence.

Reports of related and unexpected SAEs will be submitted to the REC within 15 dats of the CI becoming aware of the event, using the 'report of serious adverse event form' for non-CTIMPs published on the National Research Ethics Service (NRES) website.

5.5 Software for statistical analysis

SPSS, Prism or 'R'

5.6 Sample size

As detailed above.

- 10 of patients commencing Denosumab
- 10 of patients commencing other anti-resorptive medication

5.7 Management and delivery

Shauna Culshaw and colleagues will manage and analyse study data.

6 Study closure/ Definition of end of trial

The study will end when the CI deems that one or more of the following situations applies:

I) The planned sample size has been achieved;
II) Recruitment is so poor that completion of the study cannot reasonably be anticipated.

7. DATA HANDLING

Case report forms

Patient data will be collected at the time of the sample collection and recorded on the CRF. At the time of the sample collection, each sample will be assigned a code, recorded on the CRF. All data associated with analysis of the samples will be collected and stored linked only to the code. The data linking the patient code with the patient data will be stored securely on University of Glasgow computers, using password protected file encryption. Hard copies will be stored in a locked file at the University of Glasgow Dental School. The data recording sample analysis will include only the patient code and will also be stored on University of Glasgow computers. Hard copies of this data will be stored in laboratory record books, kept at the University of Glasgow Dental School. Samples will be stored appropriate to the type of sample, within facilities at the University of Glasgow, with a detailed description of the sample location, use(s) and ultimately disposal, documented in a sample log record. Any data changes will be recorded in order to maintain a complete audit trail (reason for change, data change made, who made change).

Record retention

To enable evaluations and/or audits from regulatory authorities, the investigator agrees to keep records, including the identity of all participating subject (sufficient information to link records), all original signed informed consent forms, serious adverse event reports, source documents, and detailed records of treatment disposition in accordance with ICH GCP, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. Data will be retained by the research team for 15 years.

8 Trial Management

Routine management of trial: Trial Management Group

The study will be coordinated from the University of Glasgow Dental Hospital, by the Trial Management Group. The Trial Management Group will include the CI, statistician, research nurse and clinical and laboratory investigators involved in the collection and use of clinical samples. The group will monitor all aspects of the conduct and progress of the study, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the study itself.

9 Study Audit

Study site-set up and site file will be provided once the study has received R&D approval. Study audit visits will be conducted according to NHS Greater Glasgow and Clyde audit processes. Additional visits may be undertaken in required. Investigators and site staff will be notified in advance of any audit visits.

10 Protocol Amendments

Any change in the study protocol will require an amendment. Any proposed protocol amendments will be initiated by the CI following discussion with the Sponsor to determine whether an amendment is non-substantial or substantial. All amended versions of the protocol will be signed by the CI and Sponsor representative. Before the amended protocol can be implemented, favourable opinion/approval must be sought from the original reviewing REC and R&D office.

11 Ethical Considerations

11.1 Ethical conduct of the study

The study will be carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and its revisions (Tokyo [1975], Venice [1983], Hong Kong [1989], South Africa [1996] and Edinburgh [2000]).

Favourable ethical opinion will be sought from appropriate REC before patients are entered into this research study. Patients will only be allowed to enter the study once they have provided written informed consent.

The CI will be responsible for updating the REC of any new information related to the study.

11.2 Informed consent

Written informed consent should be obtained from each trial participant. Only patients able to consent for themselves will be included in the study. The consultant or his/her representative, or the research nurse will explain the exact nature of the study in writing, provision of patient information sheet, and verbally. This will include the known side-effects, for example, some mild discomfort from measuring PPD (as is standard in any routine dental exam), that may be experienced. Trial participants will be informed that they are free to withdraw their consent from the study or study examinations and sample collections at any time, and it will be emphasised to participants that their decision to participate in the study does NOT influence the treatment they receive in any way.

12 Insurance and indemnity

The study is sponsored by NHS Greater Glasgow and Clyde. The sponsor will be liable for negligent harm caused by the design of the trial. NHS indemnity is provided under the Clinical Negligence and Other Risks Indemnity Scheme (CNORIS).

The NHS has a duty of care to patients treated and examined, whether or not the patient is taking part in a clinical trial, and the NHS remains liable for clinical negligence and other negligent harm to patients under its duty of care.

13 Funding

NHSR&D Endowment - further applications pending preliminary data.

14 Annual Reports

Reports to the funder will be coordinated by the university. Annual reports will be submitted to the REC and sponsor with the first submitted one year after the data that all trial related approvals are in place.

15 Dissemination of findings

Findings will be presented at group meetings, seminars at Glasgow Dental School and at the Institute of Infection, Immunity and Inflammation. In addition, findings will be presented at national and international meetings and submitted to peer review journals. Public engagement in research is important to the investigators and funders. Therefore, outreach activities such as ‘meet the expert’ at Glasgow Science Centre will include findings from these studies and significant findings of broader interest will be promoted with advice and support from the University of Glasgow press office to publicise any key developments and to engage with a wider audience.

Appendix 1B
Patient information and consent documentation

Periodontal Health in patients on Denosumab *Version 1.2 15th May 2020*

Information Sheet
We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?
Professor Shauna Culshaw
University of Glasgow Dental School.
378 Sauchiehall Street,
Glasgow G2 3JZ, Telephone 0141 211 9795.

What is the purpose of the study?
The purpose of this study is to learn about the effects of medications used to treat osteoporosis such as Denosumab (Prolia®) or Bisphosphonates (for example Alendronic Acid) on your mouth. During the study we will collect saliva, gingival crevicular fluid and plaque samples before your bone medication is started and again between 4 and 12 weeks after you have started your bone medication.

Osteoporosis
Osteoporosis or 'brittle bone disease' is a condition when your bones become more prone to fracture as they are weaker due to a change in the cells you have which create and take away bone. If you want to know more about osteoporosis talk to the doctor or nurse who is treating you. Osteoporosis is sometimes treated with medication to slow down the cells that change your bone.

Why have I been invited?
You have been invited to take part in this study as you have attended the Bone Metabolism Clinic and are starting to take medications for your bones.

Do I have to take part?
No, it is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide not to take part in this study, your care will continue to be provided at the Bone Metabolism Clinic you attended today. You can stop taking part at any time in this study without giving a reason. The dentist or dental nurse from the research team can advise you about any concerns you may have. If you decide to stop taking part in the study and you have samples of your saliva, plaque and gingival crevicular fluid stored with us, you can ask to have these destroyed or you may wish to consider the samples being used as they have already been obtained. A decision to not take part at any time will not affect the standard of care you receive.

What does taking part involve?
Not everyone may be able to take part in this study. We are looking for patients who have 16 or more teeth. We would like to look at your gums and to takes samples of your dental plaque, saliva, and fluid that covers your teeth. We would like to take these samples before you start your medication and once more 4-12 weeks after starting.

Saliva sample
You will be asked to donate some saliva by dribbling into a tube for 2-4 minutes.

Gingival crevicular fluid sample:
A very small volume of fluid (gingival crevicular fluid 'GCF') normally travels from the margins of your gums across your teeth, coating your teeth with the fluid. We wish to collect this fluid by placing a small collection device on your tooth and allowing the fluid to collect. The fluid will take a few minutes to accumulate in the collection device. You should not experience any discomfort and there are no risks associated with this procedure.

Bacterial plaque sample
A small amount of dental plaque will be collected from below your gum. First, the plaque above the gum will be removed (as is routinely done during a 'scale and polish'). We wish to collect a sample of the bacteria from just below the gum. The bacteria will be collected using an instrument normally used for cleaning below the gum. The bacteria will be placed in fluid so they may be stored for analysis at a later date. The removal of the bacteria from below the gum to collect the sample will be identical to removal of bacteria for a normal 'scale and polish'. You should not experience any discomfort and there are no risks associated with this procedure.

Patient Visits
If you consent, you will get a full examination of your mouth, including a detailed examination of your gum health. This study will require you to visit the Queen Elizabeth University Hospital or Glasgow Dental Hospital one or two more times. You can choose which location is more convenient for you. We will give you detailed advice about how best to clean your teeth to a very high standard. We will ask to collect the dental plaque, gingival crevicular fluid and saliva samples immediately after the dental exam. So that everybody in the study is cleaning their teeth in a similar way, we will provide you with an electric toothbrush. We then ask you to return to the clinic 4-12 weeks after you start the medication for your bones. The dental exam and sample collection will take approximately 20 minutes and will be carried out in the location most suitable for you. We will reimburse travel expenses for any extra visits to the clinic (public transport or parking costs). A receipt will need to be provided for claiming travel expenses. If you need any dental treatment we can help arrange this. We can write to your dentist or can arrange treatment at Glasgow Dental Hospital.

What Will Happen To The Samples and Information I Give?
All samples will be coded and identified by your unique study number. Samples will be stored in a secure fashion at the University of Glasgow. Access to samples will be restricted to the researchers and the scientists who will analyse the samples. Samples will be stored in line with NHS Greater Glasgow and Clyde policies. Samples may be later transferred to laboratories both within the University of Glasgow and laboratories who can help us with the analysis both within and out with the UK. Samples may be shared with other collaborators within the UK and outside the UK. All samples provided to collaborators will not be labeled with any personal information about you and the samples cannot be linked to you. We also seek your permission to perform further studies on these samples (in a strictly anonymous fashion) in the future for studies designed to improve care for those with periodontal disease or osteoporosis. We do not plan any genetic testing for this study but similarly seek your permission to do this in the future (again in a strictly anonymous fashion) where it may help in studies designed to

improve care for those with periodontal disease or osteoporosis. Any further studies using these samples would only take place after further review by a Research Ethics Committee.

What are the possible benefits of taking part?
The information we get from this study may help us better understand the effects of osteoporosis medication on the mouth. This may help to improve treatment for patients who suffer from gum disease, and may help patients taking medications for osteoporosis keep their mouths healthy. You will be offered detailed oral hygiene advice to help improve your overall oral health.

What will happen to the results of the study?
You will not receive results from your analysed samples. If we see any evidence of dental disease, you will be made aware of this and if eligible will be offered treatment free of charge at Glasgow Dental Hospital. We hope the whole study will be completed in 36 months and be published in a medical journal thereafter. If you would like further information on the results of the study you can contact the Chief Investigator, Shauna Culshaw at the end of the study. Your identity and any personal details will be kept confidential. No named information about you will be published in any report of this study.

Who has reviewed the study?
This study has been reviewed and approved by the National Research Ethics Service.

Sponsor
NHS Greater Glasgow and Clyde

If you have any further questions?
We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study please contact Prof Shauna Culshaw (details above). If you would to speak to someone not closely linked to the study, please contact:

[redacted]

If you have a complaint about any aspect of the study?
If you are unhappy about the way you have been approached or treated during the study, please talk to the research team dentist or nurse. If you are still unhappy, or if you wish to complain, please use the normal NHS complaints process. If you are harmed because of someone's negligence, then you may be able to take legal action.

Thank you very much for your time.

General Data Protection Regulation

Data Transparency

NHS Greater Glasgow and Clyde is the sponsor for this study based in Glasgow Dental Hospital. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. NHS Greater Glasgow and Clyde will keep identifiable information about you up to 15 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting Dr Shauna Culshaw on 0141 211 9795.

Your Information

NHS Greater Glasgow and Clyde will collect information from you and your medical records for this research study in accordance with our instructions.

NHS Greater Glasgow and Clyde will use your name, Community Hospital Indicator (CHI) number and contact telephone number to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from NHS Greater Glasgow and Clyde and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Glasgow Dental Hospital will pass these details to NHS Greater Glasgow and Clyde along with the information collected from you. The only people in NHS Greater Glasgow and Clyde who will have access to information that identifies you will be people who need to contact you to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, CHI number or contact details.

NHS Greater Glasgow and Clyde will keep identifiable information about you from this study for up to 15 years after the study has finished.

Publication of Information

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

Your information could be used for research in any aspect of health or care, and could be combined with information about you from other sources held by researchers, the NHS or government.

Where this information could identify you, the information will be held securely with strict arrangements about who can access the information. The information will only be used for the purpose of health and care research, or to contact you about future opportunities to participate in research. It will not be used to make decisions about future services available to you, such as insurance.

Where there is a risk that you can be identified your data will only be used in research that has been independently reviewed by an ethics committee.

Periodontal Health in patients on Denosumab (PoD) (Version 1.1, 22JULY2022)

CONSENT FORM – DENTAL EXAMINATION AND SAMPLE COLLECTION

Patient Identification Number for this Study .....

1. I confirm that I have read and understand the information sheet and GDPR statement Version:_____ Date _____ for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. *Initials* ☐

1. I understand my participation is voluntary and that I am free to withdraw at any time, without giving any reason. My medical or dental care, or legal rights being affected.

2. I consent to allow the following samples to be taken for research purposes at the initial baseline appointment and at a subsequent appointment 4-6 months after the study begins:

.i) saliva

ii) plaque

iii) gingival crevicular fluid

3. I agree to allow gene analysis of my tissues for research purposes.

4. I agree to my anonymous samples of fluid/tissues being stored and used in future research projects.

I agree to my anonymous samples of fluid/tissues being stored and used in future research projects, including for genetic analysis.

I agree to my anonymous samples of fluid/tissue being stored and used in future research projects, in work with other researchers within and outside the UK.

5. I confirm I have received a signed copy of this information and consent form to keep

One copy to be retained by patient, one copy to be placed in the patients notes and one copy to be retained in study file.

8. I agree to take part in the above study. *Initials* ☐

_____	_____	_____	
Name of Patient	Signature	Date	
_____	_____	_____	
Researcher	Signature		Date

One copy to be retained by patient, one copy to be placed in the patients' notes and one copy to be retained in study file.

Appendix 1C: references for clinical study

MHRA Drug Safety Updates
(a) Medicines and Healthcare products Regulatory Agency (MHRA), 2020. *Denosumab 60 mg (Prolia): increased risk of multiple vertebral fractures after stopping or delaying ongoing treatment*. Drug Safety Update, 26 August 2020.
Available at: https://www.gov.uk/drug-safety-update/denosumab-60mg-prolia-increased-risk-of-multiple-vertebral-fractures-after-stopping-or-delaying-ongoing-treatment
(b)
Medicines and Healthcare products Regulatory Agency (MHRA), 2022. *Denosumab 60 mg (Prolia): reminder of risk of multiple vertebral fractures after stopping or delaying treatment*. Drug Safety Update, January 2022.
Available at: https://www.gov.uk/drug-safety-update/denosumab-60mg-prolia-reminder-of-risk-of-multiple-vertebral-fractures-after-stopping-or-delaying-treatment

Tsourdi et al. (2020) – European Calcified Tissue Society position statement
Tsourdi, E., Zillikens, M.C., Meier, C., Body, J.J., González-Rodríguez, E., Anastasilakis, A.D., Abrahamsen, B., McCloskey, E., Hofbauer, L.C., Guañabens, N., Obermayer-Pietsch, B., Ralston, S.H., Eastell, R., Pepe, J., Palermo, A. and Langdahl, B., 2020. *Fracture risk and management of discontinuation of denosumab therapy: a systematic review and position statement by the European Calcified Tissue Society (ECTS). Journal of Clinical Endocrinology & Metabolism*, 106(1), pp.264–281.
doi: 10.1210/clinem/dgaa756.

National Osteoporosis Guideline Group (NOGG, 2024)
National Osteoporosis Guideline Group (NOGG), 2024. *Clinical guideline for the prevention and treatment of osteoporosis.* Updated December 2024. Sheffield: NOGG / Royal Osteoporosis Society.
Available at: https://www.nogg.org.uk/sites/nogg/download/NOGG-Guideline-2024.pdf [Accessed 5 October 2025].

Cummings et al. (2018; 2022) – FREEDOM post-hoc analyses
Cummings, S.R., Ferrari, S., Eastell, R., Gilchrist, N., Beck-Jensen, J.E., McClung, M., Roux, C., Törring, O., Valter, I., Wang, A.T. and Brown, J.P., 2018. *Vertebral fractures after discontinuation of denosumab: a post-hoc analysis of the randomized placebo-controlled FREEDOM trial and its extension. Journal of Bone and Mineral Research*, 33(2), pp.190–198.
doi: 10.1002/jbmr.3337.

Cummings, S.R., Ferrari, S., Brown, J.P., McClung, M.R., Eastell, R., Gilchrist, N., et al., 2022. *Multiple vertebral fractures after denosumab discontinuation: FREEDOM and FREEDOM Extension trials additional post-hoc analyses.Journal of Bone and Mineral Research*, 37(10), pp.1945–1954.
doi: 10.1002/jbmr.4663.

Long-term denosumab efficacy and safety studies

Bone, H.G., Bolognese, M.A., Yuen, C.K., Kendler, D.L., Miller, P.D., Yang, Y.C., Lewiecki, E.M. and the FREEDOM Extension Investigators, 2017. *Ten years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomized FREEDOM trial and open-label extension. The Lancet Diabetes & Endocrinology*, 5(7), pp.513–523.
doi: 10.1016/S2213-8587(17)30159-6.

Brown, J.P., Roux, C., Törring, O., Ho, P.R., Beck Jensen, J.E., Gilchrist, N., Recknor, C.P., Austin, M., Wang, A., Wagman, R.B. and Bone, H.G., 2014. *Effects of up to 5 years of denosumab treatment on bone histology and histomorphometry: the FREEDOM study extension. Journal of Bone and Mineral Research*, 29(9), pp.2051–2056.
doi: 10.1002/jbmr.2236.

Appendix 1D: References for clinical protocol submitted for grant/study approval

1. Eke PI, Dye BA, Wei L, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol*. 2015;86(5):611-622. doi:10.1902/jop.2015.140520.
2. Bostanci N, Saygan B, Emingil G, et al. Adult Dental Health Survey 2009 - Summary report and thematic series - NHS Digital. *J Clin Periodontol*. 2013;85(1):81-95. doi:10.1007/s12160-013-9486-6.
3. Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. *J Clin Periodontol*. 1981;8(4):281-294. doi:10.1111/j.1600-051X.1981.tb02039.x.
4.. Kerr NW. Treatment of chronic periodontitis. 45% failure rate after 5 years. *Br Dent J*. 1981;150(8):222-224. doi:10.1038/sj.bdj.4804579.5.
6. Listl S, Galloway J, Mossey PA, Marcenes W. Global economic impact of dental diseases. In: *Journal of Dental Research*. Vol 94. ; 2015:1355-1361. doi:10.1177/0022034515602879.
7. Malcolm J, Awang RA, Oliver-Bell J, et al. IL-33 Exacerbates Periodontal Disease through Induction of RANKL. *J Dent Res*. 2015;94(7):968-975. doi:10.1177/0022034515577815.
8. Malcolm J, Millington O, Millhouse E, et al. Mast cells contribute to porphyromonas gingivalis- induced bone loss. *J Dent Res*. 2016;95(6):704-710. doi:10.1177/0022034516634630.
9. Oliver-Bell J, Butcher JP, Malcolm J, et al. Periodontitis in the absence of B cells and specific anti-bacterial antibody. *Mol Oral Microbiol*. 2015;30(2):160-169. doi:10.1111/omi.12082.
10. Han X, Lin X, Yu X, et al. Porphyromonas gingivalis infection-associated periodontal bone resorption is dependent on receptor activator of NF-κB ligand. *Infect Immun*. 2013. doi:10.1128/IAI.00043-13.
11. Kawai T, Matsuyama T, Hosokawa Y, et al. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *Am J Pathol*. 2006;169(3):987-998. 10.2353/ajpath.2006.060180.

Appendix 2: Abbreviations

PUBMED	Public Medline
LILACS	Latin American and Caribbean Literature on Health Sciences
CDC-AAP	Centre for Disease Control and Prevention & American Academy of Periodontoloty
CPI	Community Periodontal Index
NHS	National Health Services – The United Kingdom
USD	United States Dollars
IL	InterLeukin
IL1RA	InterLeukin 1 Receptor antagonist
TNF α	Tumour Necrosis Factor Alpha
RANK	Receptor Activator of Nuclear Factor kappa-Β
RANKL	Receptor Activator of Nuclear Factor kappa-Β Ligand
sRANKL	Soluble Receptor Activator of Nuclear Factor kappa-Β Ligand
OPG	Osteoprotegerin
PDL	Periodontal Ligament
ALP	Alkaline Phosphatase
ELISA	Enzyme Linked Immuno Sorbent Assay
GCF	Gingival Crevicular Fluid
pmol/l	Pico mol / litre
Pg/ml	Picogram/millilitre
Lys3	Lysine Gene
ASN	Aminoacyl tRNA Synthetase
STAT3	Signal Transducer and Activator of Transcription (It's a protein that plays a crucial role in cell signalling particularly in response to cytokines and growth factors)
CXCL	C-X-C motif Chemokine Ligand
CXCR	C-X-C Chemokine Receptor
DMARDs	Disease Modifying Anti-Rheumatic Drugs
RA	Rheumatoid Arthritis
PUFA	Poly Unsaturated Fatty Acids
FREEDOM	Fracture Reduction Evaluation of Denosumab in Osteoporosis – every 6 months
HALT	Hormone Ablation Therapy for breast and prostate Cancer
HMT	Host Modulation Therapy

Appendix 3: Data management plan

Data Management Plan template for PGR students - Drug Repurposing In Dentistry

1. Overview	
Student name	Sridhar N Rao
Supervisor name	Prof. Shauna Culshaw and Dr. Andrea Sherriff
Project title	Drug Repurposing in Dentistry
Funder & award number	NHSGGC R&d
Project Summary	This is a pilot feasibility/acceptability study of patients starting on anti resorptive medication for osteoporosis e.g. Denosumab and Bisphosphonates. Bone remodelling is mediated by RANK (Receptor Activator for Nuclear Factor kß)- RANKL (Receptor Activator for Nuclear Factor kß Ligand) -OPG (OsteoProtegerin) axis. Bone loss due to Periodontitis (gum infection) or Osteoporosis results when bone loss supersedes bone formation. Denosumab, is a specific human monoclonal antibody to RANKL, acts by blocking RANKL and reducing bone loss. My study was started with an aim to identify whether anti-bone resorptive medication specifically Denosumab affects inflammation and bone loss in the periodontium. The study comprises of a clinical and a laboratory component

2a. Data – Clinical Data
What types of data will be collected or created?
• Quantitative Data of experimental measurements from clinical components • Photographs of the armamentarium (not patient)
What formats will you use?
• Data in spread sheets will be in .csv/.xls format • Image stored as .tiff files
How much data will you collect?
About 2 GB

2. Data – Laboratory Data
What types of data will be collected or created?
• Quantitative Data of levels of RANKL and OPG as determined by the xMAP reader. • Photographs of the armamentarium and procedural photographs
What formats will you use?
• Data in spread sheets will be in .csv/.xls format • Images stored as .tiff files
How much data will you collect?

About 4 GB

3. Documentation for 2a and 2b
How will the data be documented and described?
Numerical data from stored and collected clinical samples - Salivary samples from healthy (MVLS ethics study), Gingivitis (MVLS ethics study), Periodontitis (IRAPT 40 study) and Patients on Denosumab (POD study). The nomenclature of the anonymised patient begins with the study name followed by an alphabet and or number of the patient. For example MVLS H 01/ IR 01/POD 01. The samples are demographically matched to have an unbiased representation. (Will be detailed in a README file)
Are there any standards for this in your field of research?
Good Clinical Practice

4. Ethics and Intellectual Property
Who owns the data in your project?
Sridhar N Rao, Prof. Shauna Culshaw and Dr. Andrea Sherriff
Detail any ethical, legal or commercial considerations relating to your research data
My project will collect clinical and laboratory data related to human subjects – who will be pseudonymised and will not be identifiable for the research team.
How will these concerns be dealt with?
Ethical approval has been gained from NHS ethics committee and data will be processed in line with legal requirements (data protection/GDPR). As regards to using the stored samples the consent has been given to use the human tissue for research (from patients participating in the studies) there is no legal requirement to obtain ethical approval for research carried out on licensed premises (- Use of human tissue in research - Health Research Authority (hra.nhs.uk)- Last updated on 16 Nov 2021) Clinical and Laboratory data from patients are pseudonymised and no longer identifiable for the research team. The patient data is archived and only accessible to the main supervisor Prof. Shauna Culshaw via stored data on a separate system.

5. Storage and organisation
How will the data be named, organised and structured?
a. Clinical Study – All files will start with POD (Patients On Denosumab) followed by enrolment number and date specimen taken in DDMMYYYY format (pseudonymised)and stored as after scanning as .rtf and the excel spread sheet of the clinical values as .xls /.csv. The pseudonymised patients identity will be used for future purposes.

b. Laboratory investigations – will be on an excel spread sheet in .xls/.csv format and named as ddmonYYPOD Lab Results Masterfile.
How will the data be stored for the duration of the project?
One drive for Business https://www.gla.ac.uk/myglasgow/office365/ University of Glasgow.
How will the data be backed up during the project?
One drive for Business https://www.gla.ac.uk/myglasgow/office365/ University of Glasgow -will be backed up and named as mentioned in 5 b. ddmonYYPODupdateLab or ddmonYYPODupdateResult
Does access to the data need to be controlled for the duration of the project?
Most of the personal information will be pseudonymised and stored on OneDrive from the University of Glasgow.- this ensures maintenance of confidentiality.The access to these files will be encrypted and password protected. Physical measures like locking the screen when away from computer and locking the office door when leaving the office. Transfer of files will happen Via University of Glasgow OneDrive.
Who has the right to access the data during the project?
Sridhar N Rao (PGR student) , Prof. Shauna Culshaw (Supervisor) and Dr. Andrea Sherriff (Supervisor)

6. Deposit and long-term preservation
Which data should be retained long-term?
The Clinical and Laboratory will be held for about 10 years.
How long will data be retained for?
Minimum of 10 years – as per University of Glasgow requirement.
Where will the data be archived at the end of the project?
Enlighten: Research Data, The University of Glasgow's Institutional data repository or University of Glasgow Onedrive which will enable the data to be curated effectively.
What formats will the data be archived in?
.xls/.csv and .tiff

7. Data sharing
Is any of the data suitable for sharing?
Not suitable for sharing as of now.
How will the data be shared?
n/a
Who should be able to access and use the shared data?
n/a

8. Implementation
Who is responsible for implementing this plan?
Sridhar N Rao (PGR student) , Prof. Shauna Culshaw (Supervisor) and Dr. Andrea Sherriff (Supervisor)
How will this plan be kept up-to-date?

The Plan will be reviewed on a regular basis with Prof. Culshaw (supervisor) and kept up to date
What actions are necessary to implement this plan?
With the help of local IT support
What training or further information are needed to implement this plan?
n/a

Chapter 5: References

AALTONEN KJ, YLIKYLÄ S, TUULIKKI JOENSUU J, ISOMÄKI P, PIRILÄ L, KAUPPI M, RANNIO T, EKLUND K, BLOM M, NORDSTRÖM D. Efficacy and effectiveness of tumour necrosis factor inhibitors in the treatment of rheumatoid arthritis in randomized controlled trials and routine clinical practice. Rheumatology (Oxford). 2017 May 1;56(5):725-735. doi: 10.1093/rheumatology/kew467. PMID: 28064209.

ABUSLEME, L., HOARE, A., HONG, B. Y. & DIAZ, P. I. 2021. Microbial signatures of health, gingivitis, and periodontitis. *Periodontol 2000,* 86**,** 57-78.

AL HABASHNEH, R., ALCHALABI, H., KHADER, Y. S., HAZZA'A, A. M., ODAT, Z. & JOHNSON, G. K. 2010. Association between periodontal disease and osteoporosis in postmenopausal women in jordan. *J Periodontol,* 81**,** 1613-21.

AMIRI MA, AMIRI D, HAMEDANI S. 2024. Thermosensitive Hydrogels for Periodontal Regeneration: A Systematic Review of the Evidence. Clin Exp Dent Res. 2024 Dec;10(6):e70029. doi: 10.1002/cre2.70029. PMID: 39539029; PMCID: PMC11561135.

ARIAS-BUJANDA, N., REGUEIRA-IGLESIAS, A., BALSA-CASTRO, C., NIBALI, L., DONOS, N. & TOMAS, I. 2020. Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: A systematic review and meta-analysis. *J Clin Periodontol,* 47**,** 2-18.

ASIF, S., AHMAD, B., HAMZA, S. A., TAIB, H., KASSIM, N. K. & ZAINUDDIN, S. L. A. 2022. Investigation of Salivary RANKL and OPG Levels in Periodontitis Patients at Hospital Universiti Sains Malaysia. *Eur J Dent,* 16**,** 173-178.

BAIMA G, ARCE M, ROMANDINI M, VAN DYKE T. Inflammatory and Immunological Basis of Periodontal Diseases. J Periodontal Res. 2025 Oct 9. doi: 10.1111/jre.70040. Epub ahead of print. PMID: 41065279.

BALTA, M. G., PAPATHANASIOU, E., BLIX, I. J. & VAN DYKE, T. E. 2021. Host Modulation and Treatment of Periodontal Disease. *J Dent Res,* 100**,** 798-809.

BELIBASAKIS, G. N. & BOSTANCI, N. 2012. The RANKL-OPG system in clinical periodontology. *J Clin Periodontol,* 39**,** 239-48.

BERGLUNDH T, DONATI M, ZITZMANN N. B cells in periodontitis: friends or enemies? Periodontol 2000. 2007;45:51-66. doi: 10.1111/j.1600-0757.2007.00223.x. PMID: 17850448

BONE HG, WAGMAN RB, BRANDI ML, BROWN JP, CHAPURLAT R, CUMMINGS SR, CZERWIŃSKI E, FAHRLEITNER-PAMMER A, KENDLER DL, LIPPUNER K, REGINSTER JY, ROUX C, MALOUF J, BRADLEY MN, DAIZADEH NS, WANG A, DAKIN P, PANNACCIULLI N, DEMPSTER DW, PAPAPOULOS S. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol. 2017 Jul;5(7):513-523. doi: 10.1016/S2213-8587(17)30138-9. Epub 2017 May 22. PMID: 28546097.

BOSTANCI, N., ILGENLI, T., EMINGIL, G., AFACAN, B., HAN, B., TOZ, H., ATILLA, G., HUGHES, F. J. & BELIBASAKIS, G. N. 2007. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *J Clin Periodontol,* 34**,** 370-6.

BOSTANCI, N., SAYGAN, B., EMINGIL, G., ATILLA, G. & BELIBASAKIS, G. N. 2011. Effect of periodontal treatment on receptor activator of NF-kappaB ligand

and osteoprotegerin levels and relative ratio in gingival crevicular fluid. *J Clin Periodontol,* 38**,** 428-33.
BOTELHO, J., MACHADO, V., LEIRA, Y., PROENCA, L., CHAMBRONE, L. & MENDES, J. J. 2022. Economic burden of periodontitis in the United States and Europe: An updated estimation. *J Periodontol,* 93**,** 373-379.
BOYCE BF, XING L. Biology of RANK, RANKL, and osteoprotegerin. Arthritis Res Ther. 2007;9 Suppl 1(Suppl 1):S1. doi: 10.1186/ar2165. PMID: 17634140; PMCID: PMC1924516.
BUDUNELI, N., BIYIKOGLU, B., SHERRABEH, S. & LAPPIN, D. F. 2008. Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. *J Clin Periodontol,* 35**,** 846-52.
CALDEIRA FID, HIDALGO MAR, DE CARLI DIAS ML, SCAREL-CAMINAGA RM, PIGOSSI SC. Systematic review of ratios between disease /health periodontitis modulators and meta-analysis of their levels in gingival tissue and biological fluids. Arch Oral Biol. 2021 Jul;127:105147. doi: 10.1016/j.archoralbio.2021.105147. Epub 2021 May 18. PMID: 34044319
CALVANO KUCHLER, E., MASCHIETTO PUCINELLI, C., CARPIO HORTA, K., ASSED BEZERRA DA SILVA, R., DE CASTRO COSTA, M., REZENDE VIEIRA, A., NELSON-FILHO, P., ASSED BEZERRA DA SILVA, L., SANTOS ANTUNES, L. & AZEREDO ANTUNES, L. 2020. Dental Caries, Developmental Defects of Enamel and Enamel Microhardness Associated with Genetic Polymorphisms in the RANK/RANKL/OPG System. *J Clin Pediatr Dent,* 44**,** 35-40.
CAMPBELL L, MILLHOUSE E, MALCOLM J, CULSHAW S. T cells, teeth and tissue destruction - what do T cells do in periodontal disease? Mol Oral Microbiol. 2016 Dec;31(6):445-456. doi: 10.1111/omi.12144. Epub 2015 Dec 2. PMID: 26505640.
CARVALHO, R., BOTELHO, J., MACHADO, V., MASCARENHAS, P., ALCOFORADO, G., MENDES, J. J. & CHAMBRONE, L. 2021. Predictors of tooth loss during long-term periodontal maintenance: An updated systematic review. *J Clin Periodontol,* 48**,** 1019-1036.
CHANG, A. M., KANTRONG, N. & DARVEAU, R. P. 2021. Maintaining homeostatic control of periodontal epithelial tissue. *Periodontol 2000,* 86**,** 188-200.
CHANG, W. P., CHANG, W. C., WU, M. S., PAI, J. T., GUO, Y. C., CHEN, K. C., LIU, M. E., CHIU, W. T. & HUNG, K. S. 2014. Population-based 5-year follow-up study in Taiwan of osteoporosis and risk of periodontitis. *J Periodontol,* 85**,** e24-30.
CHAPPLE ILC, HIRSCHFELD J, KANTARCI A, WILENSKY A, SHAPIRA L. The role of the host-Neutrophil biology. Periodontol 2000. 2023 May 18. doi: 10.1111/prd.12490. Epub ahead of print. PMID: 37199393.
CHEN Y, ZHOU T, ZHANG HH, KANG N. Bovine lactoferrin inhibits alveolar bone destruction in an orthodontic rat model with periodontitis. Ann Anat. 2021 Sep;237:151744. doi: 10.1016/j.aanat.2021.151744. Epub 2021 Apr 22. PMID: 33895284.
COCHRAN, D. L. 2008. Inflammation and bone loss in periodontal disease. *J Periodontol,* 79**,** 1569-76.
CROFT M, SALEK-ARDAKANI S, WARE CF. Targeting the TNF and TNFR superfamilies in autoimmune disease and cancer. Nat Rev Drug Discov. 2024 Dec;23(12):939-961. doi: 10.1038/s41573-024-01053-9. Epub 2024 Oct 24. PMID: 39448880.

DELIMA AJ, KARATZAS S, AMAR S, GRAVES DT. Inflammation and tissue loss caused by periodontal pathogens is reduced by interleukin-1 antagonists. J Infect Dis. 2002 Aug 15;186(4):511-6. doi: 10.1086/341778. Epub 2002 Jul 24. PMID: 12195378.

DEL REI DALTRO ROSA, C. D., DE LUNA GOMES, J. M., DANTAS DE MORAES, S. L., ARAUJO LEMOS, C. A., MINATEL, L., JUSTINO DE OLIVEIRA LIMIRIO, J. P. & PELLIZZER, E. P. 2021. Does non-surgical periodontal treatment influence on rheumatoid arthritis? A systematic review and meta-analysis. *Saudi Dent J,* 33, 795-804.

DINIZ-FREITAS, M., FERNANDEZ-FEIJOO, J., DIZ DIOS, P., POUSA, X. & LIMERES, J. 2018. Denosumab-related osteonecrosis of the jaw following non-surgical periodontal therapy: A case report. *J Clin Periodontol,* 45, 570-577.

DONOS N, CALCIOLARI E, BRUSSELAERS N, GOLDONI M, BOSTANCI N, BELIBASAKIS GN. The adjunctive use of host modulators in non-surgical periodontal therapy. A systematic review of randomized, placebo-controlled clinical studies. J Clin Periodontol. 2020 Jul;47 Suppl 22:199-238. doi: 10.1111/jcpe.13232. PMID: 31834951.

ENGEBRETSON, S., & KOCHER, T.2013. Evidence that periodontal therapy improves diabetes outcomes: A systematic review and meta-analysis. *Journal of Clinical Periodontology*. Apr;40 Suppl 14:S153–S163. doi:10.1111/jcpe.12084. PubMed: https://pubmed.ncbi.nlm.nih.gov/23627325

FELDMANN M. 2002. Development of anti-TNF therapy for rheumatoid arthritis. Nat Rev Immunol. May 2(5):364-71. doi: 10.1038/nri802.

FERENC K, BOŻEK A, GAWRON K. Immunological pathways triggered by Porphyromonas gingivalis in periodontitis: New insights into molecular mechanisms. Mol Immunol. 2025 Dec 4;189:119-132. doi: 10.1016/j.molimm.2025.12.001. Epub ahead of print. PMID: 41349496.

FRAGOULIS GE, SIEBERT S, MCINNES IB. Therapeutic Targeting of IL-17 and IL-23 Cytokines in Immune-Mediated Diseases. Annu Rev Med. 2016;67:337-53. doi: 10.1146/annurev-med-051914-021944. Epub 2015 Nov 4. PMID: 26565676.

GAFFEN SL, HAJISHENGALLIS G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. J Dent Res. 2008 Sep;87(9):817-28. doi: 10.1177/154405910808700908. PMID: 18719207; PMCID: PMC2692983.

GAFFEN SL, MOUTSOPOULOS NM. Regulation of host-microbe interactions at oral mucosal barriers by type 17 immunity. Sci Immunol. 2020 Jan 3;5(43):eaau4594. doi: 10.1126/sciimmunol.aau4594. PMID: 31901072; PMCID: PMC7068849.

GAL ZT, WETZEL EA, NAHED BV, SAYLOR PJ, HIRSCH JA, MARCISCANO AE, RAJE NS, TOBERT DG, YEE AJ, SHANKAR GM. Efficacy of bone-modifying agents in preventing vertebral complications of multiple myeloma: a systematic review and meta-analysis. Spine J. 2025 Oct 14:S1529-9430(25)00850-2. doi: 10.1016/j.spinee.2025.10.018. Epub ahead of print. PMID: 41101648.

GARLET GP, CARDOSO CR, CAMPANELLI AP, FERREIRA BR, AVILA-CAMPOS MJ, CUNHA FQ, SILVA JS. The dual role of p55 tumour necrosis factor-alpha receptor in Actinobacillus actinomycetemcomitans-induced experimental periodontitis: host protection and tissue destruction. Clin Exp Immunol. 2007

Jan;147(1):128-38. doi: 10.1111/j.1365-2249.2006.03260.x. PMID: 17177972; PMCID: PMC1810448.
GARTENMANN, S., MAIER, N., WIEDEMEIER, D. B., ATTIN, T. & SCHMIDLIN, P. 2020. Effect of Adjuvant Use of NSAID in Reducing Probing Pocket Depth in the Context of Conventional Periodontal Therapy: A Systematic Review of Randomized Trials. *Applied Sciences,* 10.
GIL-MONTOYA, J. A., GARRIDO-MARTINEZ, M., BARRIOS-RODRIGUEZ, R., RAMOS-GARCIA, P., LENOUVEL, D., MONTES-CASTILLO, C. & MARTINEZ-RAMIREZ, M. J. 2021. Association between low bone mineral density and periodontitis in generally healthy perimenopausal women. *J Periodontol,* 92**,** 95-103.
GOLUB, L. M., LEE, H. M., & RYAN, M. E.1999. Tetracyclines inhibit connective-tissue breakdown: new agents for periodontal therapy. *Annals of the New York Academy of Sciences*. 1999;878:396–410. doi:10.1111/j.1749-6632.1999.tb07691.x. PubMed: https://pubmed.ncbi.nlm.nih.gov/10415737.
GORSKI D, LAZO REL, DE SOUZA DA, BORBA HHL, PONTAROLO R, TONIN FS. Biological Therapy and Small Molecules for Adults With Crohn's Disease: Systematic Review and Network Meta-Analysis. Pharmacotherapy. 2025 Sep;45(9):587-599. doi: 10.1002/phar.70049. Epub 2025 Aug 7. PMID: 40772520; PMCID: PMC12424525.
GOYAL, L., GUPTA, S. & PERAMBUDHURU, Y. 2024. Antibiotics as adjunct to non-surgical periodontal therapy in diabetic patients. *Evid Based Dent,* 25**,** 202-203.
GRAVES, D. T.2008. Cytokines that promote periodontal tissue destruction. *Journal of Periodontology*. 2008 Aug;79(8 Suppl):1585–1591. doi:10.1902/jop.2008.080183.
PubMed: https://pubmed.ncbi.nlm.nih.gov/18673014
GUPTA V, DAWAR A, DAS SK, YADAV VS, NALWA V, HAIDRUS R, PUROHIT BM, GOYAL L. Cardiovascular Biomarkers in Periodontitis: A Systematic Review and Meta-Analysis. Oral Dis. 2025 Aug 31. doi: 10.1111/odi.70084. Epub ahead of print. PMID: 40887818.
HAJISHENGALLIS G, HAJISHENGALLIS E, KAJIKAWA T, WANG B, YANCOPOULOU D, RICKLIN D, LAMBRIS JD. Complement inhibition in pre-clinical models of periodontitis and prospects for clinical application. Semin Immunol. 2016 Jun;28(3):285-91. doi: 10.1016/j.smim.2016.03.006. Epub 2016 Mar 24. PMID: 27021500; PMCID: PMC4987193.
HAJISHENGALLIS G, CHAVAKIS T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol. 2021 Jul;21(7):426-440. doi: 10.1038/s41577-020-00488-6. Epub 2021 Jan 28. PMID: 33510490; PMCID: PMC7841384.
HAJISHENGALLIS G, HASTURK H, LAMBRIS JD; Contributing authors. C3-targeted therapy in periodontal disease: moving closer to the clinic. Trends Immunol. 2021 Oct;42(10):856-864. doi: 10.1016/j.it.2021.08.001. Epub 2021 Sep 2. PMID: 34483038; PMCID: PMC8487962.
HAJISHENGALLIS, G. & LAMONT, R. J. 2021. Polymicrobial communities in periodontal disease: Their quasi-organismal nature and dialogue with the host. *Periodontol 2000,* 86**,** 210-230.
HAJISHENGALLIS G, LI X, DIVARIS K, CHAVAKIS T. Maladaptive trained immunity and clonal hematopoiesis as potential mechanistic links between periodontitis and inflammatory comorbidities. Periodontol 2000. 2022 Jun;89(1):215-230.

doi: 10.1111/prd.12421. Epub 2022 Mar 4. PMID: 35244943; PMCID: PMC9018499.
HATHAWAY-SCHRADER, J. D. & NOVINCE, C. M. 2021. Maintaining homeostatic control of periodontal bone tissue. *Periodontol 2000,* 86**,** 157-187.
HASTURK H, KANTARCI A, OHIRA T, ARITA M, EBRAHIMI N, CHIANG N, PETASIS NA, LEVY BD, SERHAN CN, VAN DYKE TE. RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis. FASEB J. 2006 Feb;20(2):401-3. doi: 10.1096/fj.05-4724fje. Epub 2005 Dec 22. PMID: 16373400.
HASTURK H, HAJISHENGALLIS G; LAMBRIS JD, MASTELLOS DC, YANCOPOULOU D. 2021. Phase IIa clinical trial of complement C3 inhibitor AMY-101 in adults with periodontal inflammation. J Clin Invest. 2021 Dec 1;131(23):e152973. doi: 10.1172/JCI152973. PMID: 34618684; PMCID: PMC8631591.
HASTURK H, SCHULTE F, MARTINS M, SHERZAI H, FLOROS C, CUGINI M, CHIU CJ, HARDT M, VAN DYKE T. Safety and Preliminary Efficacy of a Novel Host-Modulatory Therapy for Reducing Gingival Inflammation. Front Immunol. 2021 Sep 13;12:704163. doi: 10.3389/fimmu.2021.704163. PMID: 34589083; PMCID: PMC8475270.
HERRERA D, MATESANZ P, MARTÍN C, OUD V, FERES M, TEUGHELS W. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy: A systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:239-256. doi: 10.1111/jcpe.13230. PMID: 31912531.
HONG, S. J., YANG, B. E., YOO, D. M., KIM, S. J., CHOI, H. G. & BYUN, S. H. 2021. Analysis of the relationship between periodontitis and osteoporosis/fractures: a cross-sectional study. *BMC Oral Health,* 21**,** 125.
HOU, J. M., XUE, Y. & LIN, Q. M. 2012. Bovine lactoferrin improves bone mass and microstructure in ovariectomized rats via OPG/RANKL/RANK pathway. *Acta Pharmacol Sin,* 33**,** 1277-84.
JEAN WACTAWSKI-WENDE, S. G. G. M. T. R. J. G. M. T. R. G. D., , ALEX W. HO,, ERNEST HAUSMANN, MYROSLAW M. HRESHCHYSHYN 1996. The Role of Osteopenia in Oral Bone Loss and Periodontal Disease. *Journal of Periodontology,* 67**,** 1076-1084.
JEFFCOAT, M. K., ET AL.1998.Adjunctive use of a subgingival controlled-release chlorhexidine chip (PerioChip). *Journal of Periodontology*. 1998 Nov;69(11):1254–1261.
JEFFCOAT, M. 2005. The Association Between Osteoporosis and Oral Bone Loss. *Journal of Periodontology,* 76**,** 2125-2132.
JOHNSTON W, PATERSON M, PIELA K, DAVISON E, SIMPSON A, GOULDING M, RAMAGE G, SHERRIFF A, CULSHAW S. The systemic inflammatory response following hand instrumentation versus ultrasonic instrumentation-A randomized controlled trial. J Clin Periodontol. 2020 Sep;47(9):1087-1097. doi: 10.1111/jcpe.13342. Epub 2020 Jul 27. PMID: 32628781.
JOSEPH, S. & CURTIS, M. A. 2021. Microbial transitions from health to disease. *Periodontol 2000,* 86**,** 201-209.
KADHIRESAN RATHINASAMY1, A. U., SHANMUGAPRIYA RAMAMURTHY3, RATHINAVELU GANESAN4, PARTHIBAN SAKET5, SWARNA ALAMELU 2020. Estimation of TNF-α Levels in Saliva and Serum of Patients with Periodontal Health and Chronic Periodontitis: A Case-control Study. *J Contemp Dent Pract,* 21**,** 148-151.
KARIMBUX NY, SARAIYA VM, ELANGOVAN S, ALLAREDDY V, KINNUNEN T, KORNMAN KS, DUFF GW. Interleukin-1 gene polymorphisms and chronic periodontitis in

adult whites: a systematic review and meta-analysis. J Periodontol. 2012 Nov;83(11):1407-19. doi: 10.1902/jop.2012.110655. Epub 2012 Feb 21. Erratum in: J Periodontol. 2013 Feb;84(2):273. PMID: 22348697.

KAUR, K., DHINGRA, S., SLEZAK, J., SHARMA, A. K., BAJAJ, A. & SINGAL, P. K. 2009. Biology of TNFalpha and IL-10, and their imbalance in heart failure. *Heart Fail Rev,* 14**,** 113-23.

KEESTRA, J. A., GROSJEAN, I., COUCKE, W., QUIRYNEN, M. & TEUGHELS, W. 2015. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated chronic periodontitis: a systematic review and meta-analysis. *J Periodontal Res,* 50**,** 294-314.

KENDLER, D. L., COSMAN, F., STAD, R. K. & FERRARI, S. 2022. Denosumab in the Treatment of Osteoporosis: 10 Years Later: A Narrative Review. *Adv Ther,* 39**,** 58-74.

KERSCHBAUMER A, SEPRIANO A, BERGSTRA SA, SMOLEN JS, VAN DER HEIJDE D, CAPORALI R, EDWARDS CJ, VERSCHUEREN P, DE SOUZA S, POPE JE, TAKEUCHI T, HYRICH KL, WINTHROP KL, ALETAHA D, STAMM TA, SCHOONES JW, LANDEWÉ RBM. Efficacy of synthetic and biological DMARDs: a systematic literature review informing the 2022 update of the EULAR recommendations for the management of rheumatoid arthritis. Ann Rheum Dis. 2023 Jan;82(1):95-106. doi: 10.1136/ard-2022-223365. Epub 2022 Nov 11. PMID: 36368906.

KHATTRI S, KUMBARGERE NAGRAJ S, ARORA A, EACHEMPATI P, KUSUM CK, BHAT KG, JOHNSON TM, LODI G. Adjunctive systemic antimicrobials for the non-surgical treatment of periodontitis. Cochrane Database Syst Rev. 2020 Nov 16;11(11):CD012568. doi: 10.1002/14651858.CD012568.pub2. PMID: 33197289; PMCID: PMC9166531.

KHOSLA, S. & HOFBAUER, L. C. 2017. Osteoporosis treatment: recent developments and ongoing challenges. *Lancet Diabetes Endocrinol,* 5**,** 898-907.

KIM, B., CHO, Y. J. & LIM, W. 2021. Osteoporosis therapies and their mechanisms of action (Review). *Exp Ther Med,* 22**,** 1379.

KINANE DF, LAPPIN DF, CULSHAW S. The role of acquired host immunity in periodontal diseases. Periodontol 2000. 2024 Apr 20. doi: 10.1111/prd.12562. Epub ahead of print. PMID: 38641953.

KINANE D, BOUCHARD P; Group E of European Workshop on Periodontology. Periodontal diseases and health: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontol. 2008 Sep;35(8 Suppl):333-7. doi: 10.1111/j.1600-051X.2008.01278.x. PMID: 18724860.

KORNMAN KS. Host modulation as a therapeutic strategy in the treatment of periodontal disease. Clin Infect Dis. 1999 Mar;28(3):520-6. doi: 10.1086/515165. PMID: 10194070.

KURITANI, M., SAKAI, N., KARAKAWA, A., ISAWA, M., CHATANI, M., NEGISHI-KOGA, T., FUNATSU, T. & TAKAMI, M. 2018. Anti-mouse RANKL Antibodies Inhibit Alveolar Bone Destruction in Periodontitis Model Mice. *Biol Pharm Bull,* 41**,** 637-643.

LEE CT, LI R, ZHU L, TRIBBLE GD, ZHENG WJ, FERGUSON B, MADDIPATI KR, ANGELOV N, VAN DYKE TE. Subgingival Microbiome and Specialized Pro-Resolving Lipid Mediator Pathway Profiles Are Correlated in Periodontal Inflammation. Front Immunol. 2021 Jun 10;12:691216. doi: 10.3389/fimmu.2021.691216. PMID: 34177951; PMCID: PMC8222734.

LIM, W. H., LIU, B., MAH, S. J., CHEN, S. & HELMS, J. A. 2014. The molecular and cellular effects of ageing on the periodontal ligament. *J Clin Periodontol,* 41**,** 935-42.

LIN, D., LI, L., SUN, Y., WANG, W., WANG, X., YE, Y., CHEN, X. & XU, Y. 2014. IL-17 regulates the expressions of RANKL and OPG in human periodontal ligament cells via TRAF6/TBK1-JNK/NF-kappaB pathways. *Immunology,* 144**,** 472-85.

LIN J, BI L, YU X, KAWAI T, TAUBMAN MA, SHEN B, HAN X. Porphyromonas gingivalis exacerbates ligature-induced, RANKL-dependent alveolar bone resorption via differential regulation of Toll-like receptor 2 (TLR2) and TLR4. Infect Immun. 2014 Oct;82(10):4127-34. doi: 10.1128/IAI.02084-14. Epub 2014 Jul 21. PMID: 25047844; PMCID: PMC4187858.

LIU C, ZHANG S, BAI H, ZHANG Y, JIANG Y, YANG Z, XU X, DING Y. Soy isoflavones alleviate periodontal destruction in ovariectomized rats. J Periodontal Res. 2022 Jun;57(3):519-532. doi: 10.1111/jre.12981. Epub 2022 Feb 25. PMID: 35212419.

LOOS, B. G. & NEEDLEMAN, I. 2020. Endpoints of active periodontal therapy. *Journal of Clinical Periodontology,* 47**,** 61-71.

LOPEZ ROLDAN, A., GARCIA GIMENEZ, J. L. & ALPISTE ILLUECA, F. 2020. Impact of periodontal treatment on the RANKL/OPG ratio in crevicular fluid. *PLoS One,* 15**,** e0227757.

LOPEZ-OLIVA I, MALCOLM J, CULSHAW S. Periodontitis and rheumatoid arthritis-Global efforts to untangle two complex diseases. Periodontol 2000. 2024 Feb 27. doi: 10.1111/prd.12530. Epub ahead of print. PMID: 38411247.

MAEKAWA T, HOSUR K, ABE T, KANTARCI A, ZIOGAS A, WANG B, VAN DYKE TE, CHAVAKIS T, HAJISHENGALLIS G. Antagonistic effects of IL-17 and D-resolvins on endothelial Del-1 expression through a GSK-3ß-C/EBPß pathway. Nat Commun. 2015 Sep 16;6:8272. doi: 10.1038/ncomms9272. PMID: 26374165; PMCID: PMC4573473.

MAHENDRA, J. 2020. Cytokines: The Immune Warriors in Periodontal Health and Disease- An Overview. *International Journal of Pharmaceutical Research,* 13.

MALCOLM J, AWANG RA, OLIVER-BELL J, BUTCHER JP, CAMPBELL L, ADRADOS PLANELL A, LAPPIN DF, FUKADA SY, NILE CJ, LIEW FY, CULSHAW S. IL-33 Exacerbates Periodontal Disease through Induction of RANKL. J Dent Res. 2015 Jul;94(7):968-75. doi: 10.1177/0022034515577815. Epub 2015 Mar 25. PMID: 25808546.

MALCOLM J, CULSHAW S. Aberrant immunity in the oral cavity-a link with rheumatoid arthritis? Front Oral Health. 2024 Jun 14;5:1430886. doi: 10.3389/froh.2024.1430886. PMID: 38948089; PMCID: PMC11211539.

MARTINEZ-MAESTRE, M. A., GONZALEZ-CEJUDO, C., MACHUCA, G., TORREJON, R. & CASTELO-BRANCO, C. 2010. Periodontitis and osteoporosis: a systematic review. *Climacteric,* 13**,** 523-9.

MARRUGANTI C, SUVAN JE, D'AIUTO F. Periodontitis and metabolic diseases (diabetes and obesity): Tackling multimorbidity. Periodontol 2000. 2023 Oct 16. doi: 10.1111/prd.12536. Epub ahead of print. PMID: 37845800.

MATTHEW T. DRAKE, B. L. C., , AND SUNDEEP KHOSLA, 2008. Bisphosphonates: Mechanism of Action and Role in Clinical Practice. *Mayo Clin Proc.,* 83**,** 1032-1045.

MF TIMMERMAN, G. V. D. W. 2006. Risk factors for periodontitis. *International Journal of Dental Hygiene,* 4**,** 2-7.

MYNENI, S. R., BROCAVICH, K. & H, H. W. 2020. Biological strategies for the prevention of periodontal disease: Probiotics and vaccines. *Periodontol 2000,* 84, 161-175.
NAKANO M, YOSHIDA A, WAKABAYASHI H, TANAKA M, YAMAUCHI K, ABE F, MASUDA Y. Effect of tablets containing lactoferrin and lactoperoxidase on gingival health in adults: A randomized, double-blind, placebo-controlled clinical trial. J Periodontal Res. 2019 Dec;54(6):702-708. doi: 10.1111/jre.12679. Epub 2019 Jul 10. PMID: 31292969.
NARAYANAN, P. 2013. Denosumab: A comprehensive review. *South Asian J Cancer,* 2, 272-7.
NAYAK S, GREENSPAN SL. A systematic review and meta-analysis of sequential treatment strategies for osteoporosis. Osteoporos Int. 2025 Oct 17. doi: 10.1007/s00198-025-07717-5. Epub ahead of print. Erratum in: Osteoporos Int. 2025 Nov 18. doi: 10.1007/s00198-025-07751-3. PMID: 41105226.
NEPRELYUK OA, ZHAD'KO SI, ROMANENKO IG, KRIVENTSOV MA. Adjunctive use of omega-3 fatty acids in combination with low-dose aspirin in periodontitis: Systematic review and meta-analysis. J Periodontal Res. 2023 Dec;58(6):1128-1138. doi: 10.1111/jre.13191. Epub 2023 Oct 3. PMID: 37787044.
NESSE W, ABBAS F, VAN DER PLOEG I, SPIJKERVET FK, DIJKSTRA PU, VISSINK A. Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol. 2008 Aug;35(8):668-73. doi: 10.1111/j.1600-051X.2008.01249.x. Epub 2008 Jun 28. PMID: 18564145.
NEURATH, N. & KESTING, M. 2024. Cytokines in gingivitis and periodontitis: from pathogenesis to therapeutic targets. *Front Immunol,* 15, 1435054.
NICOLOPOULOS K, MOSHI MR, STRINGER D, MA N, JENAL M, VREUGDENBURG T. The clinical effectiveness of denosumab (Prolia®) in patients with hormone-sensitive cancer receiving endocrine therapy, compared to bisphosphonates, selective estrogen receptor modulators (SERM), and placebo: a systematic review and network meta-analysis. Arch Osteoporos. 2023 Jan 10;18(1):18. doi: 10.1007/s11657-023-01211-3. PMID: 36624318.
NILE CJ, SHERRABEH S, RAMAGE G, LAPPIN DF. Comparison of circulating tumour necrosis factor superfamily cytokines in periodontitis patients undergoing supportive therapy: a case-controlled cross-sectional study comparing smokers and non-smokers in health and disease. J Clin Periodontol. 2013 Sep;40(9):875-82. doi: 10.1111/jcpe.12134. PMID: 23919740.
O'NEILL SM, LEAHY J, HARTE M, KANE D, EAMES H, NGUYEN V, O'LEARY A, MC CONNELL D, MC CULLAGH L, FLYNN C, ADAMS R, CLARKE M. The efficacy and safety of Janus kinase inhibitors in adults with rheumatoid arthritis: a systematic review and cumulative meta-analysis. Semin Arthritis Rheum. 2025 Dec;75:152885. doi: 10.1016/j.semarthrit.2025.152885. Epub 2025 Nov 22. PMID: 41330174.
OCHANJI, A. A., MATU, N. K. & MULLI, T. K. 2017. Association of salivary RANKL and osteoprotegerin levels with periodontal health. *Clin Exp Dent Res,* 3, 45-50.
OKAMOTO, K., NAKASHIMA, T., SHINOHARA, M., NEGISHI-KOGA, T., KOMATSU, N., TERASHIMA, A., SAWA, S., NITTA, T. & TAKAYANAGI, H. 2017. Osteoimmunology: The Conceptual Framework Unifying the Immune and Skeletal Systems. *Physiol Rev,* 97, 1295-1349.
ONO, T., HAYASHI, M., SASAKI, F. & NAKASHIMA, T. 2020. RANKL biology: bone metabolism, the immune system, and beyond. *Inflamm Regen,* 40, 2.

ORTIZ, P., et al.2009. Periodontal therapy reduces the severity of active rheumatoid arthritis: a randomized clinical trial. *Journal of Periodontology*. 2009;80(4):535–542. PubMed: https://pubmed.ncbi.nlm.nih.gov/19335072

PATEL, S. K., et al.2021 Biorelevant and screening dissolution methods for PLGA microspheres: relevance to local delivery systems. *Pharmaceutics*. 2021;13(4):550.doi:10.3390/pharmaceutics13040550. PubMed: https://pubmed.ncbi.nlm.nih.gov/33486044

PETIT C, CULSHAW S, WEIGER R, HUCK O, SAHRMANN P. Impact of treatment of rheumatoid arthritis on periodontal disease: A review. Mol Oral Microbiol. 2024 Aug;39(4):199-224. doi: 10.1111/omi.12454. Epub 2024 Feb 16. PMID: 38363058.

PRADEEP, A. R., & THORAT, M. S.2010. Clinical effect of subgingivally delivered simvastatin in chronic periodontitis: randomized clinical trial. *Journal of Periodontology*. 2010;81:214–222. doi:10.1902/jop.2009.090701. PubMed: https://pubmed.ncbi.nlm.nih.gov/20151799

PLOTKIN, L. I. & BRUZZANITI, A. 2019. Molecular signaling in bone cells: Regulation of cell differentiation and survival. *Adv Protein Chem Struct Biol,* 116**,** 237-281.

PRESHAW, P. M.2008. Host-modulation therapy: current status and future directions. *Periodontology 2000*. 2008;47:131–147. doi:10.1111/j.1600-0757.2008.00252.x. PubMed: https://pubmed.ncbi.nlm.nih.gov/18715360

PRESHAW PM, TAYLOR JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? J Clin Periodontol. 2011 Mar;38 Suppl 11:60-84. doi: 10.1111/j.1600-051X.2010.01671.x. PMID: 21323705.

PRESHAW PM, BISSETT SM. Periodontitis and diabetes. Br Dent J. 2019 Oct;227(7):577-584. doi: 10.1038/s41415-019-0794-5. PMID: 31605062.

RAHNAMA, M., JASTRZEBSKA-JAMROGIEWICZ, I., JAMROGIEWICZ, R., NOGALSKI, A. & JAGIELAK, M. 2013. Influence of hormone replacement therapy on osteoprotegerin and receptor activator of nuclear factor kappa-B ligand concentrations in menopausal women. *J Interferon Cytokine Res,* 33**,** 485-92.

RAJAGOPAL, A. & ANAND, M. 2022. THE ROLE OF STATINS IN PERIODONTAL THERAPY – A REVIEW. *Cumhuriyet Dental Journal*, 25**,** 200-205.

RAKIC, M., LEKOVIC, V., NIKOLIC-JAKOBA, N., VOJVODIC, D., PETKOVIC-CURCIN, A. & SANZ, M. 2013. Bone loss biomarkers associated with peri-implantitis. A cross-sectional study. *Clin Oral Implants Res,* 24**,** 1110-6.

RAKIC M, CALCIOLARI E, GRANT MM, RADOVANOVIC S, BOSTANCI N, PRESHAW PM. Host Markers of Periodontal Diseases: Meta-Analysis of Diagnostic Accuracy Studies. J Clin Periodontol. 2025 Aug;52 Suppl 29:155-181. doi: 10.1111/jcpe.14167. Epub 2025 Apr 19. PMID: 40251882.

RAMADAN, D. E., HARIYANI, N., INDRAWATI, R., RIDWAN, R. D. & DIYATRI, I. 2020. Cytokines and Chemokines in Periodontitis. *Eur J Dent,* 14**,** 483-495.

RAO, N. S., PRADEEP, A. R., KUMARI, M., et al.2013. Simvastatin local drug delivery in smokers with chronic periodontitis: a randomized controlled clinical trial. *Journal of Periodontology*. 2013;84(5):597–605. PubMed: https://pubmed.ncbi.nlm.nih.gov/23713634

RELVAS, M., SILVESTRE, R., GONCALVES, M., CABRAL, C., MENDES-FRIAS, A., MONTEIRO, L. & VIANA DA COSTA, A. 2023. Analysis of Salivary Levels of IL-

1β, IL17A, OPG and RANK-L in Periodontitis Using the 2017 Classification of Periodontal Diseases-An Exploratory Observational Study. *J Clin Med,* 12.

REN B, LU J, LI M, ZOU X, LIU Y, WANG C, WANG L.2021 Anti-inflammatory effect of IL-1ra-loaded dextran/PLGA microspheres on Porphyromonas gingivalis lipopolysaccharide-stimulated macrophages in vitro and in vivo in a rat model of periodontitis. Biomed Pharmacother. 2021 Feb;134:111171. doi: 10.1016/j.biopha.2020.111171. Epub 2020 Dec 28. PMID: 33383312

REN, J., FOK, M. R., ZHANG, Y., HAN, B. & LIN, Y. 2023. The role of non-steroidal anti-inflammatory drugs as adjuncts to periodontal treatment and in periodontal regeneration. *J Transl Med,* 21**,** 149.

SAHNI V, VAN DYKE TE. Immunomodulation of periodontitis with SPMs. Front Oral Health. 2023 Oct 20;4:1288722. doi: 10.3389/froh.2023.1288722. PMID: 37927821; PMCID: PMC10623003.

SANZ, M., HERRERA, D., KEBSCHULL, M., CHAPPLE, I., JEPSEN, S., BEGLUNDH, T., SCULEAN, A., TONETTI, M. S., PARTICIPANTS, E. F. P. W. & METHODOLOGICAL, C. 2020. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. *J Clin Periodontol,* 47 Suppl 22**,** 4-60.

SIEBERT S, TSOUKAS A, ROBERTSON J, MCINNES I. Cytokines as therapeutic targets in rheumatoid arthritis and other inflammatory diseases. Pharmacol Rev. 2015;67(2):280-309. doi: 10.1124/pr.114.009639. PMID: 25697599.

SMOLEN, J. S., ALETAHA, D., & MCINNES, I. B. Rheumatoid arthritis. *The Lancet*. 2016 Oct 22;388(10055):2023–2038. doi:10.1016/S0140-6736(16)30173-8.
PubMed: https://pubmed.ncbi.nlm.nih.gov/27156434

SMOLEN JS, LANDEWÉ RBM, BERGSTRA SA, KERSCHBAUMER A, SEPRIANO A, ALETAHA D, CAPORALI R, EDWARDS CJ, HYRICH KL, POPE JE, DE SOUZA S, STAMM TA, TAKEUCHI T, VERSCHUEREN P, WINTHROP KL, BALSA A, BATHON JM, BUCH MH, BURMESTER GR, BUTTGEREIT F, CARDIEL MH, CHATZIDIONYSIOU K, CODREANU C, CUTOLO M, DEN BROEDER AA, EL AOUFY K, FINCKH A, FONSECA JE, GOTTENBERG JE, HAAVARDSHOLM EA, IAGNOCCO A, LAUPER K, LI Z, MCINNES IB, MYSLER EF, NASH P, POOR G, RISTIC GG, RIVELLESE F, RUBBERT-ROTH A, SCHULZE-KOOPS H, STOILOV N, STRANGFELD A, VAN DER HELM-VAN MIL A, VAN DUUREN E, VLIET VLIELAND TPM, WESTHOVENS R, VAN DER HEIJDE D. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. Ann Rheum Dis. 2023 Jan;82(1):3-18. doi: 10.1136/ard-2022-223356. Epub 2022 Nov 10. Erratum in: Ann Rheum Dis. 2023 Mar;82(3):e76. doi: 10.1136/ard-2022-223356corr1. PMID: 36357155.

SUN, J., ZHENG, Y., BIAN, X., GE, H., WANG, J. & ZHANG, Z. 2021. Non-surgical periodontal treatment improves rheumatoid arthritis disease activity: a meta-analysis. *Clin Oral Investig,* 25**,** 4975-4985.

TEEUW WJ, SLOT DE, SUSANTO H, GERDES VE, ABBAS F, D'AIUTO F, KASTELEIN JJ, LOOS BG. Treatment of periodontitis improves the atherosclerotic profile: a systematic review and meta-analysis. J Clin Periodontol. 2014 Jan;41(1):70-9. doi: 10.1111/jcpe.12171. Epub 2013 Oct 29. PMID: 24111886.

TELES, R. P., & TELES, F. R. F.2010. Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontitis, and the subgingival microbiota. *Journal of Periodontology*. 2010;81:436–447.

doi:10.1902/jop.2009.090397.
PubMed: https://pubmed.ncbi.nlm.nih.gov/20059421
TELLA, S. H. & GALLAGHER, J. C. 2014. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol,* 142, 155-70.
TENORIO, J. R., DUARTE, N. T., ANDRADE, N. S., BERGAMINI, M. L., MAMANA, A. C., BRAZ-SILVA, P. H. & ORTEGA, K. L. 2022. Assessment of bone metabolism biomarkers in serum and saliva of cirrhotic patients. *Clin Oral Investig,* 26, 1861-1868.
TEODORESCU, A. C., MARTU, I., TESLARU, S., KAPPENBERG-NITESCU, D. C., GORIUC, A., LUCHIAN, I., MARTU, M. A., SOLOMON, S. M. & MARTU, S. 2019. Assessment of Salivary Levels of RANKL and OPG in Aggressive versus Chronic Periodontitis. *J Immunol Res,* 2019, 6195258.
TEZAL, M., WACTAWSKI-WENDE, J., GROSSI, S. G., HO, A. W., DUNFORD, R. & GENCO, R. J. 2000. The Relationship Between Bone Mineral Density and Periodontitis in Postmenopausal Women. *Journal of Periodontology,* 71, 1492-1498.
TOBON-ARROYAVE, S. I., ISAZA-GUZMAN, D. M., RESTREPO-CADAVID, E. M., ZAPATA-MOLINA, S. M. & MARTINEZ-PABON, M. C. 2012. Association of salivary levels of the bone remodelling regulators sRANKL and OPG with periodontal clinical status. *J Clin Periodontol,* 39, 1132-40.
TONETTI, M. S., JEPSEN, S., JIN, L. & OTOMO-CORGEL, J. 2017. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *J Clin Periodontol,* 44, 456-462.
TRINDADE, D., CARVALHO, R., MACHADO, V., CHAMBRONE, L., MENDES, J. J. & BOTELHO, J. 2023. Prevalence of periodontitis in dentate people between 2011 and 2020: A systematic review and meta-analysis of epidemiological studies. *J Clin Periodontol,* 50, 604-626.
TROMBELLI, L., FARINA, R., POLLARD, A., CLAYDON, N., FRANCESCHETTI, G., KHAN, I. & WEST, N. 2020. Efficacy of alternative or additional methods to professional mechanical plaque removal during supportive periodontal therapy: A systematic review and meta-analysis. *J Clin Periodontol,* 47 Suppl 22, 144-154.
USUI, M., ONIZUKA, S., SATO, T., KOKABU, S., ARIYOSHI, W. & NAKASHIMA, K. 2021. Mechanism of alveolar bone destruction in periodontitis - Periodontal bacteria and inflammation. *Jpn Dent Sci Rev,* 57, 201-208.
VILLORIA GEM, FISCHER RG, TINOCO EMB, MEYLE J, LOOS BG. Periodontal disease: A systemic condition. Periodontol 2000. 2024 Oct;96(1):7-19. doi: 10.1111/prd.12616. Epub 2024 Nov 4. PMID: 39494478; PMCID: PMC11579822.
W. S. SIMONET, D. L. L. C. R. D., M. KELLEY, M.-S. CHANG, R. LU¨THY, H. Q. NGUYEN, S. WOODEN, L. BENNETT, T. BOONE,, G. SHIMAMOTO, M. D. & R. ELLIOTT, A. C., H.-L. TAN, . TRAIL, J. SULLIVAN, E. DAVY, N. BUCAY, L. RENSHAW-GEGG, T. M. HUGHES, D. HILL, W. PATTISON, P. CAMPBELL, S. SANDER, G. VAN, J. TARPLEY, P. DERBY, R. LEE, AMGEN EST PROGRAM, AND W. J. BOYLE 1997. Osteoprotegerin: A Novel Secreted Protein Involved in the Regulation of Bone Density. *Cell,* 89, 309-319.
WARA-ASWAPATI, N., SURARIT, R., CHAYASADOM, A., BOCH, J. A. & PITIPHAT, W. 2007. RANKL upregulation associated with periodontitis and Porphyromonas gingivalis. *J Periodontol,* 78, 1062-9.

WEST, N., CHAPPLE, I., CLAYDON, N., D'AIUTO, F., DONOS, N., IDE, M., NEEDLEMAN, I., KEBSCHULL, M., BRITISH SOCIETY OF, P. & IMPLANT DENTISTRY GUIDELINE GROUP, P. 2021. BSP implementation of European S3 - level evidence-based treatment guidelines for stage I-III periodontitis in UK clinical practice. *J Dent,* 106**,** 103562.

WILLIAMS, R. C., et al. 2002. Treatment of periodontitis by local administration of minocycline microspheres. *Journal of Periodontology*. 2002 May;73(5):533–542.

YAMAMOTO, M. & AIZAWA, R. 2021. Maintaining a protective state for human periodontal tissue. *Periodontol 2000,* 86**,** 142-156.

YASUDA, H. 2021. Discovery of the RANKL/RANK/OPG system. *J Bone Miner Metab,* 39**,** 2-11.

ZHAO, H., HU, J. & ZHAO, L. 2021. The effect of low-level laser therapy as an adjunct to periodontal surgery in the management of postoperative pain and wound healing: a systematic review and meta-analysis. *Lasers Med Sci,* 36**,** 175-187.

ZHOU S, DONG X, CHEN C, MA L, WU Y, ZHOU Y, CUI Y. Efficacy and Safety of Eculizumab for Paroxysmal Nocturnal Hemoglobinuria: A Systematic Review and Meta-Analysis. J Pediatr Hematol Oncol. 2021 Aug 1;43(6):203-210. doi: 10.1097/MPH.0000000000002178. PMID: 33902068.