

The Role of *Treponema denticola* in the Periodontitis Alveolar Bone Damage: A Systematic Review

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ABSTRACT

Background: Periodontitis is a chronic inflammation condition of the periodontal tissue, which causes irreversible, progressive deterioration of the tooth-supporting tissue, leading to tooth loss. One of the main bacteria in the pathogenesis of periodontitis is *Treponema denticola*. Its involvement in periodontitis includes producing and releasing various virulence factors that further modulate inflammation and ignite alveolar bone destruction. To examine *Treponema denticola*'s role in the mechanism of alveolar bone damage in periodontitis.

Method: This systematic review was conducted from August 2021 until April 2022. Qualified literature is evaluated based on inclusion criteria, including published in English within the last ten years and research articles available in full text. The inclusion literature's determination was based on the PRISMA (Preferred Reporting Item for Systematic Review and Meta-Analysis).

Result: Six journals fit the inclusion criteria and discuss *Treponema denticola* involvement in alveolar bone destruction via several mechanisms, including periplasmic flagella as its virulence factor. *Treponema denticola* can inhibit osteogenic cell differentiation and induce the production TNF- α , IL-6, and IL-1 β , which are the proinflammatory cytokines involved in osteoclastogenesis. The induction mechanism of these various cytokines can ultimately increase osteoclast differentiation by increasing RANKL expression and decreasing OPG expression.

Conclusion: *Treponema denticola* is involved in alveolar bone destruction by inhibiting bone formation and inducing an inflammatory response in immune cells that can increase osteoclast differentiation, as observed in alveolar bone destruction.

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INTRODUCTION

Periodontitis is a chronic inflammatory condition of the periodontal tissues, which can lead to progressive, irreversible damage to the supporting tissues of the teeth and can initiate tooth loss. The leading cause of periodontitis is the bacterial invasion to the periodontal tissues, and the severity of the disease is influenced by bacterial virulence factors and the host's immune response to the bacterial invasion.¹⁻³ Since periodontitis is a multifactorial disease, several risk factors such as age, gender, systemic disease, smoking, socioeconomic status, and genetics also affect the severity and the occurrence of periodontitis. Furthermore, periodontitis appears to associate with the onset and severity of some chronic systemic diseases.⁴⁻⁷

The main etiology of periodontitis is commensal bacteria found in the periodontal tissue, but due to disruption of the ecological balance or homeostasis, bacterial overgrowth occurs and forms a biofilm.⁸⁻¹⁰ *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Treponema denticola* are the primary pathogens that cause periodontitis. Treponemas (*Treponema denticola*, *Treponema lecithinolyticum*, *Treponema socranskii*) play a vital role in periodontal tissue diseases such as chronic periodontitis and acute necrotizing gingivitis, ulcerative infections from endodontics treatment, and some acute abscesses in the oral cavity. Several studies reported that *Treponema denticola* (*T. denticola*) could form and release various virulence factors, such as proteolytic enzymes and cytolytic factors involved in the pathogenesis of periodontitis. The outer membrane protein of *T. denticola* is a critical virulence factor that plays a vital role in binding bacteria to host cells during bacterial infection on periodontal tissue, hence initiating the onset of

periodontitis. One of these proteins is T cell-dependent protein (Td92) which can stimulate the production of prostaglandin E2 (PGE2) in areas of inflammation.¹¹

Bone damage occurs due to a disturbance in the balance between the activity of osteoblasts as bone-forming cells and osteoclasts as bone resorption cells. The mechanism of this damage is through the excessive production of various bacterial products and inflammatory cytokines that cause inflammation as a trigger for bone damage.⁸ Cells that play a role in inflammation, such as T cells, B cells, macrophages, and neutrophils, can increase bone loss by producing inflammatory mediators including cytokines that trigger bone resorption through the role of osteoclasts. Osteoclastogenesis mediated by cytokine products such as RANKL and OPG plays an essential role in bone resorption.¹² In addition, macrophage colony-stimulating factor (M-CSF), which is the main cytokine, has been reported to bind to the c-fms receptor, which can stimulate osteoclast differentiation by regulating osteoclast precursors signaling systems.¹³

Previous studies have demonstrated that bacteria can infect intracellular osteoclasts and proliferate within them. Bacteria can activate Nuclear Factor of Activated T cell Cytoplasmic 1 (NFATc1) and receptor activator of nuclear factor-kappa B (NF-κB) and play a role in the RANKL signaling pathway. The study explained that osteoclasts have a role in harboring bacteria during infection and facilitate a mechanism that allows bacteria to escape from the immune system, ultimately resulting in alveolar bone destruction.¹⁴ In periodontal tissue, *T. denticola* initially must pass through a barrier consisting of an epithelial layer and a basement membrane to invade the

underlying tissue. This action involves cytopathic, motility, and chemotaxis mechanisms. Furthermore, after *T. denticola* penetrates the periodontal tissue, the released Td92 can affect alveolar bone surface cells, including osteoclasts and osteoblasts.^{11,15} To date, there have been several studies reporting the role of *T. denticola* virulence factors, such as LOS and Td92, in causing alveolar bone destruction. These virulence factors control RANKL and OPG expression, which are the primary molecules responsible in osteoclastogenesis.¹²

Various studies have used different experimental protocols, culture methods, and sources of *T. denticola* and bone cells to elucidate the role of *T. denticola* in alveolar bone destruction in periodontitis. The detailed and specific mechanism of *T. denticola* involvement in bone destruction, including its interaction with the osteoclasts, is requisite to construct a new method for targeting *T. denticola*. The technique that targets specific proteins involved in alveolar bone destruction synthesized by *T. denticola* or bone cells is critical to tackling the problem of the antibiotics overused in society.

Therefore, it warrants a systematic review to collect and review all the articles related to *T. denticola* and bone cells' interaction and its effects on the onset of periodontitis. It is hoped this review can provide adequate information to be a reference in research design in determining effective and efficient periodontitis therapy targets in the future.

RESEARCH METHODS

A systematic literature search using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines was conducted on articles in journal articles found in two electronic databases, Pubmed and Scopus.

Journals published in English were published in the last ten years, from 2012 to 2022. The search was conducted using a combination of the following key terms: "*Treponema denticola*" OR "*T. denticola*" AND "bone" OR "bone loss" OR "bone destruction" OR "bone resorption" OR "bone formation" OR "bone remodeling" OR "osteoblasts" OR "osteoclasts" OR "osteocytes". The selection of titles and abstracts was carried out based on the data to eliminate irrelevant articles. The selected articles would then be filtered again based on inclusion and exclusion criteria.

Prior to conducting a literature search on the Pubmed and Scopus databases, we first developed research questions for keywords. The research question was "What is the role of *Treponema denticola* bacteria in the mechanism of alveolar bone damage in periodontitis?". Later the research questions used SPIDER analysis to clarify the focus of research questions, interpretations, and summaries.^{16,17} (Supplementary Data. Table 1) The next stage was article elimination by checking for duplication in both electronic databases using Endnote software.

Assessment of the quality of selected literature was carried out using a guideline checklist. There were 11 components used to assess the title and abstract, introduction, methods, results and discussion. Each item had a score of 0 to 2, where a score of 0 indicates "not accurate/insufficient", a score of 1 indicates "incomplete/partly sufficient" and a score of 2 indicates "accurate/complete", with the title and objectives component in the introduction section only had a score of 0 to 1.¹⁸ (Supplementary Data. Table 2)

RESULT

This search yielded 80 articles via PubMed and 770 articles via Scopus. Full-text search on Scopus was

performed using the EndNote software produced 685 full-text articles were obtained on Scopus. After checking for duplication at the elimination stage, the amount of literature is 708 articles. The articles resulting from the duplication check were then filtered based on titles and abstracts adapted to the research topic, namely the analysis of the mechanism of bone damage by *T. denticola*. The articles excluded from the screening stage were 683, resulting in 25 articles that would be proceeded to the eligibility test (Figure 1).

Characteristics of Selected Literature

In the final stage of the literature search, four articles were selected. In the selected papers, the bacteria used were whole *T. denticola* or periplasmic flagella, major surface protein complex (MSPc), outer membrane vesicles (OMVs), and Td92, which are virulence factors of *T. denticola*. The target cells used were monocytes in one article,¹⁹ pre-osteoblasts in one article,²⁰ macrophages in one article,²¹ and periodontal ligament stem cells (PDLSC) in one article.²²

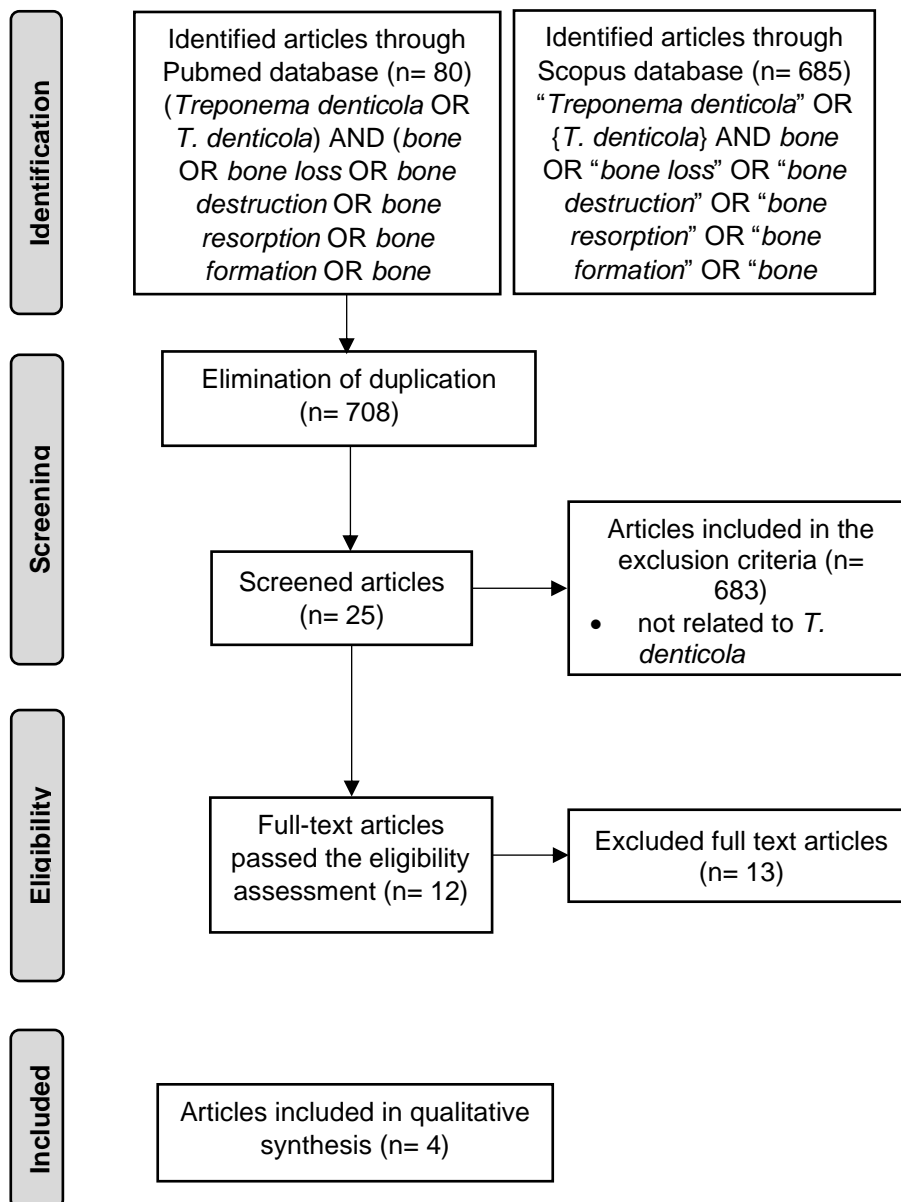


Figure 1. Flow-chart of literature screening

Table 1. Characteristic of the Eligible Articles

Authors	Bacteria/ Virulence Factors	Target Cells and Molecules	Methods	Results
Ruby et al., 2018 ¹⁹	<i>Treponema denticola</i> / Periplasmic flagella (PF)	Monocyte TNF- α , IL-1 β , IL-6, IL-10, IL- 12	<ul style="list-style-type: none"> • PF isolation and purification: The PF were isolated by vortexing with glass beads. The PF purification were analyzed by SDS-PAGE and transmission electron microscope. • Cell culture: Monocytes were purified from human peripheral blood mononuclear cells (PBMCs) with a monocyte isolation system, and the cytokine levels were analyzed by ELISA. • The recognition of <i>T. denticola</i> FlaA by human serum from PF were analyzed by western blotting. 	<ul style="list-style-type: none"> • PF deficiency in <i>T. denticola</i> cells reduces their ability to stimulate cytokine production in human monocytes. • <i>T. denticola</i> cells require PF to activate NF-κB through TLR2. • Purified PF stimulate cytokine production in human monocytes and activate NF-κB through TLR2. • Human serum reacts differentially with <i>T. denticola</i> wild type and the HL51 mutant that lacks PF.
Chow et al., 2019 ²⁰	<i>Treponema denticola</i> (whole bacteria)	Macrophage/ osteoblast-like cells TNF- α , IL-1 β	<ul style="list-style-type: none"> • Cell culture: using THP-1 human monocytic cells and osteoblast-like cells treated with heat-killed bacteria. • ELISA was used to examine the IL-1β and TNF-α released by infected macrophages. • Degree of mineralization was measured by Alizarin Red, observed by microscope, and quantify by spectrophotometric analysis. 	<ul style="list-style-type: none"> • IL-1β and TNF-α production by macrophages exposed to <i>T. denticola</i> was dramatically increased. • MG-63 cells treated with conditioned macrophage media and challenged with <i>T. denticola</i> significantly reduced alkaline phosphate activity, expression of collagen type I, osteonectin, and osteoprotegerin. • After being exposed to <i>T. denticola</i> and fed with conditioned macrophage medium, MG-63 cells drastically decreased their alkaline phosphate activity as well as the expression of osteoprotegerin, osteonectin, and collagen type I. • Up to 50% of

			osteogenic cells development and mineralization are inhibited by <i>T. denticola</i> .
			• Pre-osteoblast survival is reduced by 30% when treated with macrophages exposed to <i>T. denticola</i> .
Cecil et al., 2017 ²¹	<i>Treponema denticola</i> outer membrane vesicles (OMV)	Monocyte and Macrophage TNF- α , IL-1 β , IL-8	<ul style="list-style-type: none"> • THP-1 cells were observed for their cytokine response to bacteria by measuring the secretion of TNF-α, IL-1β, IL-8 using ELISA • OMV induced production of proinflammatory cytokines in macrophages. • In monocytes, OMV from <i>Treponema denticola</i> induced TNF-α and IL-1β secretion. • Macrophages incubated with OMV from <i>T. denticola</i> secreted TNF-α, IL-1β, and IL-8.
Ramenzoni et al., 2019 ²²	<i>Treponema denticola</i> (whole bacteria)	Periodontal ligament stem cells (PDLSC) TLR2, IL-6, IL-8	<ul style="list-style-type: none"> • Gene expressions for TLR2, TLR4, IL-6, IL-8 and IL-1β in PDLSC were analyzed by quantitative RT-PCR. • Concentrations of IL-1β and IL-6 were measured by ELISA. • Cell migration assay in PDLSC • <i>T. denticola</i> induced IL-8, IL-6 TLR 2 in PDLSC, and induces PDLSC migration.

Selected Literature Quality Assessment

From the scoring results, it was found that each article has various item scores. For the title assessment, three journals received a score of 0 because the title did not accurately describe the use of *T. denticola*. The abstract assessment was used to see if there was an accurate summary regarding the background, research objectives, methodology, results, and conclusions. For the abstract evaluation, three articles received a score of 1 because they did not meet the assessment requirements. The background was assessed

based on information related to previous research results. Two articles received a score of 2, and two articles scored 1. Research objectives were evaluated based on clear objectives or hypotheses related to the research, and all articles got a score of 1.

The assessment of methods was divided into four items. For the experimental procedures, all articles got a score of 2. For the experimental animals used in the research, one article received a score of 2 due to the sufficient and complete information. In comparison, the remaining three

articles did not receive any score since these articles did not perform an animal experiment in their research. In the experimental outcomes, all articles received a score of 2 for a sufficient and complete explanation of the function and the observed target molecules. For the statistical methods, one article received a score of 2, while three received a score of 1 due to insufficient explanation.

In the assessment of the results, two articles received a score of 1, with two articles receiving a score of 2. For the discussion interpretation assessment, one article received a score of 2, and three articles received a score of 1 due to the insufficient explanation. For the research limitation, two articles received a score of 1, and three articles scored 0. The complete list of the quality assessment is shown in Table 2.

Table 2. Quality Assessment of the Selected Literature

Studies	Item Score										
	1	2	3	4	5	6	7	8	9	10	11
Ruby et al., 2017 ¹⁹	1	1	1	1	2	-	2	1	1	2	0
Chow et al., 2019 ²⁰	0	1	2	1	2	-	2	1	1	1	0
Cecil et al., 2017 ²¹	0	1	2	1	2	2	2	1	2	1	0
Ramenzoni et al., 2019 ²²	0	2	1	1	2	-	2	2	2	1	1

DISCUSSION

When the bone niche organized by bone cells is out of sync, bone breakdown occurs.²³⁻²⁵ This is demonstrated by the unfavourable greatest rate of bone destruction by osteoclasts with normal or low bone production by osteoblast. Periodontitis, an inflammatory condition with bone destruction brought on by bacteria, one of which is *T. denticola*, is the best example of this phenomena.²⁶⁻²⁹

It is unclear how bone cells are involved in *T. denticola*'s destruction to alveolar bone. In light of this, this systematic review examined the literature on the connection and potential interactions between *T. denticola* and bone cells, as well as their role in the degeneration of bone (Figure 1, 2). Four final publications were selected from this systematic review, of which two of them focused on

monocyte and macrophage, and the other two articles focused on periodontal ligament stem cells (PDLSC) and pre-osteoblast cells, respectively.

T. denticola in the periodontal tissues is associated with inflammation caused by periodontal infection. This native oral spirochetes involves in the induction of periodontitis and later alveolar bone destruction through mechanisms that involved both immune and bone cells. Previous research has shown that *T. denticola* activates the innate immune system via TLR2. The innate immune system uses pattern recognition receptors (PRR) to recognize specific molecular patterns or motifs called pathogen-associated molecular patterns (PAMPs) on invading pathogens. PRR recognizes the structure of pathogenic bacteria primarily

associated with TLR. The presence of *T. denticola* periplasmic flagella (PF) in the periodontium would allow cellular recognition via PRR and activate the innate immune response.^{19,30}

In the study using human monocytes, the origin cells of osteoclasts, it showed that *T. denticola* can stimulate several cytokines production (TNF- α , IL-1 β , IL-6, IL-10, and IL-12), which is facilitated by PF. Nevertheless, PF is also essential to activate NF- κ B via TLR2. PF deficiency in *T. denticola* cells reduces their ability to stimulate cytokine production in human monocytes. The presence of PF in the periodontal tissues contributes to the pathogenesis of periodontal disease because TNF- α , IL-1 β , and IL-6 are essential components in the inflammation. PF provides significant PAMP in *T. denticola* so that it can stimulate pro-inflammatory cytokines that are central to the host inflammatory response in the oral disease. PFs likely contain PAMPs recognized by PRRs, and the presence of PFs in host tissues may increase bacterial seroreactivity and immunogenicity. Excessive and persistent PRR signaling through TLR2 stimulation and the presence of PAMP can lead to the induction of pro-inflammatory and osteoclastic cytokines that lead to alveolar bone resorption.² The induction of interleukin (IL-36 γ) and activation of NF- κ B also has been observed in gingival keratinocytes, that subsequently ignite gingival inflammation and periodontitis.³¹

Alveolar bone resorption that is greater than bone formation is linked to apical periodontitis. Osteoblasts produces the bone matrix and some regulatory proteins required for matrix mineralization and osteoclast differentiation. Macrophages are the central inflammatory cells responsible for initiating and maintaining the inflammatory response during apical periodontitis.

Pro-inflammatory cytokines are synthesized after bacteria or their components interact with macrophages.²⁷

In the study of Chow et al., IL-1 β and TNF- α concentrations to induce the inflammatory response have been determined. The study used MG-63 cell, a pre-osteoblast cell line that can be induced to differentiate into mature osteoblasts. When conditioned medium from *T. denticola*-infected macrophages' were used to cultivate MG-63 cells, differentiation and viability of osteogenic cells were suppressed. Pro-inflammatory cytokines produced by macrophages, such as IL-1 β and TNF- α , impaired the synthesis of alkaline phosphatase (ALP) and collagen type 1 (COL1), the major building block of bone matrix. TNF- α reduces ALP mRNA and protein directly or in combination with other growth factors such as bone morphogenetic protein (BMP)-2, 4 and IFN- γ . According to the study, osteoblast maturation is reduced and osteoclast differentiation is inhibited, which slows the repair of periapical lesions.²⁰

Several proteins in the outer membrane of *T. denticola* bacteria contribute to tissue damage in periodontal disease. Major surface protein complexes (MSPc), which are transmembrane antigens with pore-forming and adhesion activities, exert specific cytotoxic effects on cells by stimulating the release of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-10 from murine macrophages. Additionally, MSPc also induces murine erythrocyte lysis in vitro. Likewise, lipooligosaccharide (LOS) in *T. denticola* induces the release of mediators involved in the differentiation of osteoclasts from bone marrow cells. Peptidoglycan extracted from *T. denticola* was shown to promote the production of pro-inflammatory cytokines by macrophage-like cells derived from monoblastic cell lines.²¹ *T. denticola*

also involves in cytoskeletal dysfunction and MMP-2 activity by inducing RASA4 as observed in periodontal fibroblast and activate TLR2/Myd88 in human oral cells that leads to tissue destruction.^{32–}

³⁴

Bacterial outer membrane vesicles (OMVs) can migrate through host tissues, disrupt tight epithelial junctions, and present bacterial virulence factors to immune cells in the underlying tissues. A study by Cecil et al. revealed that OMVs from *T. denticola* interacted strongly with monocytes and macrophages, hence inducing phagocytosis, NF- κ B activation, cellular priming, and pro-inflammation, including the secretion of IL-1 β and TNF- α through inflammasome activation both in vitro and in vivo. In periodontitis, various bacterial virulence factors, including OMVs, are released from the subgingival plaque into the underlying connective tissue and subsequently induced a pro-inflammatory host response. OMVs of periodontal pathogenic bacteria are closed proteoliposomes composed of lipopolysaccharides, lipoproteins, nucleic acids (DNA and RNA), peptidoglycans, porins, and receptors that induce the recruitment of neutrophils and macrophages, and stimulate a potent pro-inflammatory cytokine response of various host cells. Cell culture of monocytes and macrophages incubated with OMVs from *T. denticola* can induce TNF- α , IL-1 β , and IL-8.²¹ In neutrophils and macrophages, *T. denticola* also can promote Oncostatin M cytokine release to modulate the innate immune response and promote the polymicrobial environment within the oral cavity.³⁵ Although inflammation is a crucial component of the body's defences, prolonged (persistent), excessive inflammation provides beneficial nutrients to the environment for oral pathogenic bacteria that

adhere to tooth roots in periodontal pockets. These pathogenic bacteria are generally responsible for the destruction of supporting tissue and alveolar bone, which is a sign of periodontitis.^{21,36}

Periodontal ligament stem cells (PDLSCs) play an essential role in homeostasis or periodontal tissue turnover and can be used in cell-based periodontal regenerative therapy. Supernatants from bacterial cultures of *T. denticola* containing secretions from membrane-associated LOS bacteria can induce cytokine production by mouse macrophages that hence contribute to local periodontal tissue destruction. PDLSC cells cultured with the supernatant from the *T. denticola* culture showed significantly higher motility activity at 1:300 and 1:50 dilutions. This result shows that *T. denticola* endotoxin plays a role in the motility of PDLSC, which plays a vital role in periodontal tissue repair. Additionally, it was reported that *T. denticola* endotoxin stimulates gene expression of inflammatory cytokines (IL-6, IL-8) and upregulate the expression of cell surface receptor TLR2, two to three-fold by quantitative RT-PCR method.²²

Td92 protein is one of the outer membrane proteins of *T. denticola*, which stimulates osteoclastogenesis. When *T. denticola* penetrates the periodontal tissues, Td92 secreted from *T. denticola* affects alveolar bone surface cells by suppressing the OPG expression and inducing osteoclastogenesis through prostaglandin E2 (PGE2)-mediated upregulation of RANKL. PGE2 is known to be involved in the pathogenesis of periodontitis. Consequently, Td92, as the osteoclastogenesis inducer revealed in the study by Kim et al., might play a significant role in alveolar destruction observed in periodontitis.³⁷

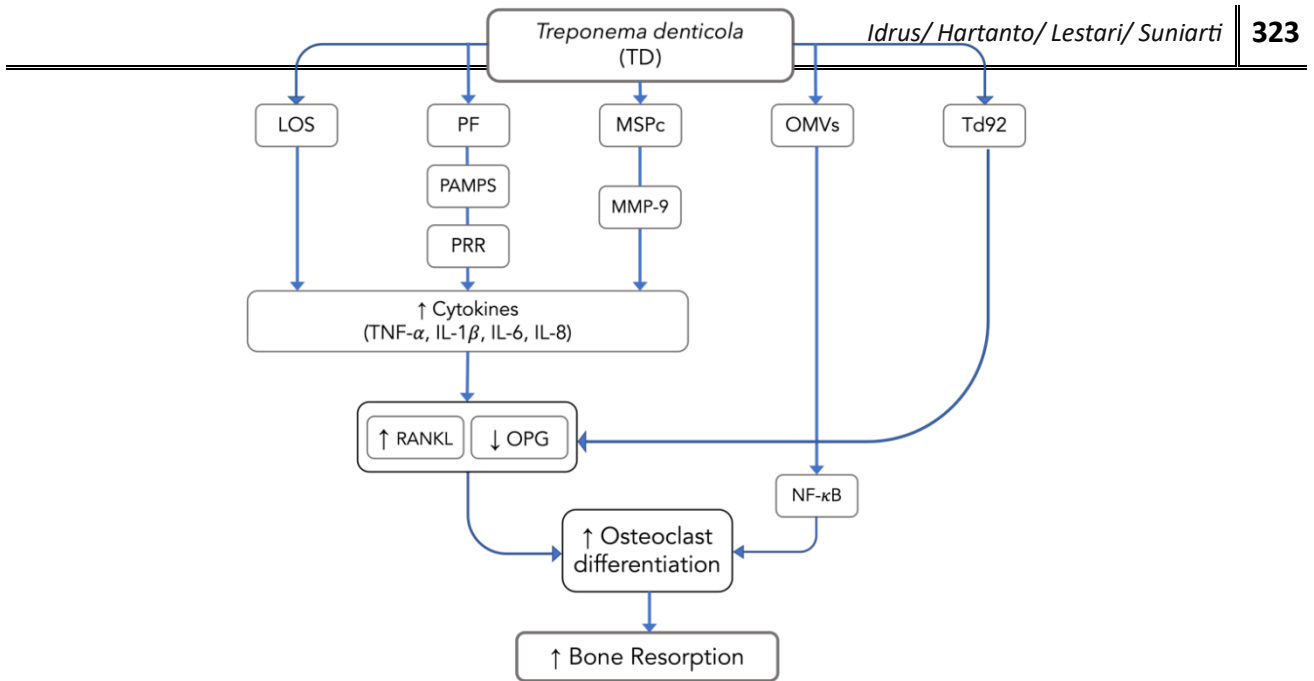


Figure 2. *Treponema denticola* mechanism of action in inducing bone resorption through its several virulence factors. (LOS: Lipooligosaccharide; PF: periplasmic flagella; PAMPS: Pathogen Associated Molecular Pattern; PRR: Pattern Recognition Receptors; MSPCs: Major Surface Protein complex; MMP-9: matrix

metalloproteinase 9; OMVs: Outer Membrane Vesicles; Td92: T cell dependent 92; NF-κB: Nuclear Factor-Kappa B; RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand; TNF-α: Tumor Necrosis Factor alpha; IL-1β/6/8: interleukin; OPG: Osteoprotegerin)

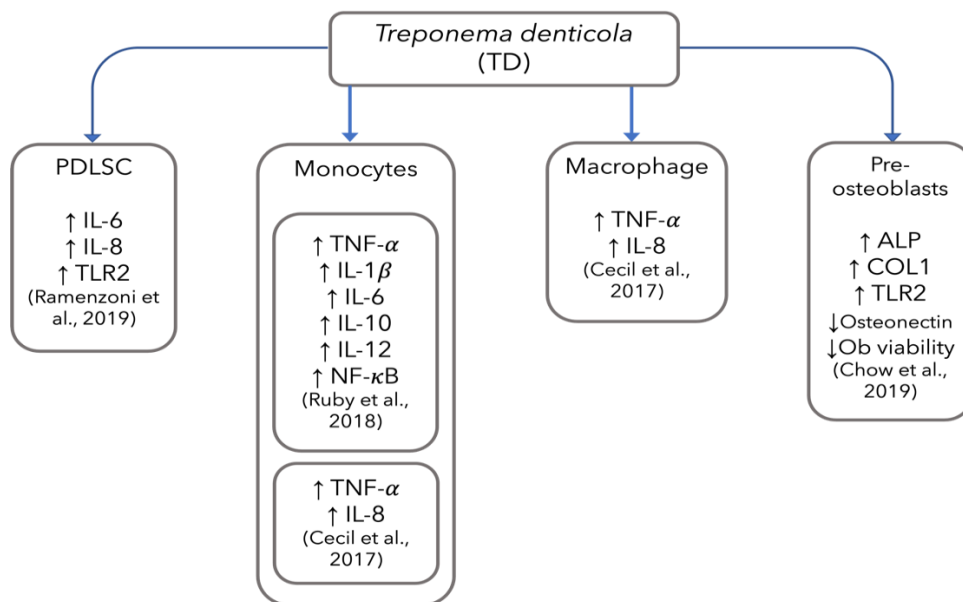


Figure 3. Interaction between *Treponema denticola*, bone cells, and their precursors cells. (NF-κB: Nuclear Factor-Kappa B; TNF-α: Tumor Necrosis Factor alpha; RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand; IL-1β/6/8/10/-

12: interleukin; OPG: Osteoprotegerin; MMP-9: matrix metalloproteinase 9; TLR-2: Toll-Like Receptors-2; ALP: alkaline phosphatase; COL1: Collagen type 1).

CONCLUSION

The four selected articles reviewed in this systematic review showed that *T. denticola* could promote the secretion of pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) on PDLSCs macrophages, monocytes, and pre-osteoblasts in periodontal tissues. *T. denticola* exerts its ability through flagella, its virulence factors, or direct bacteria-host cell contact. These mechanisms will further enhance osteoclastogenesis and lead to alveolar bone destruction initiation.

There are still limited publications on *T. denticola* in alveolar bone destruction, and information on the detailed mechanism of bone destruction through *T. denticola* interaction with bone cells remains limited. Therefore, further research is needed regarding the relationship between bacteria and bone cells, osteoclasts in particular, by assessing several aspects, such as the involvement of the virulence factors of *T. denticola* and the possible internalization of the *T. denticola* into the bone cells, which are indispensable for the alveolar bone destruction. By specifically understanding this mechanism, the novel technique in controlling *T. denticola* virulence without antibiotics would be more promising soon.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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