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REVIEW

Molecular systems architecture of host-microbiome interactions in periodontitis

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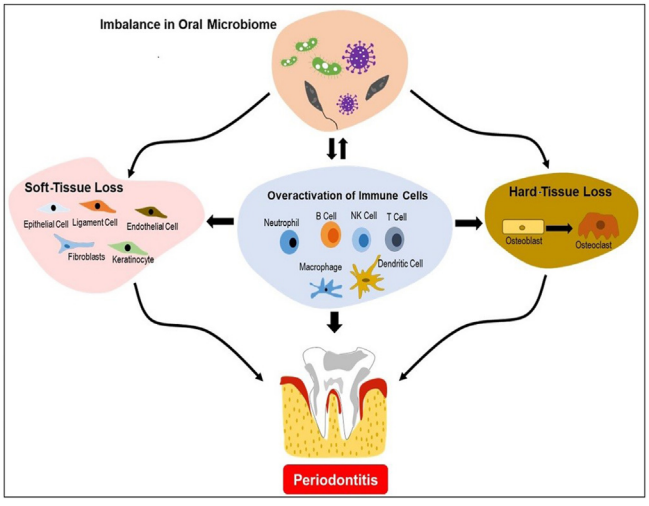

Abstract

Objectives. To develop a systems-level understanding of host-microbial interactions that lead to the pathogenesis of periodontitis. Such an understanding may identify therapeutic targets for developing efficacious treatments for periodontitis.

Search Strategy. Three databases are searched for relevant peer-reviewed articles published from January 1980 through April 2022.

Citation Sources. The citation sources include PubMed, MEDLINE, and Google Scholar.

Study Selection Criteria. The systems biology tool CytoSolve (CytoSolve) was used to perform the systematic review and to support the curation and development of the molecular systems architecture of periodontitis pathogenesis. Full-length articles that contained Medical Subject Headings key words relevant to periodontitis pathogenesis were selected for a comprehensive review.

Data Elements Included. The molecular interactions across the 8 cell types—gingival epithelial, fibroblast, periodontal ligament, endothelial, keratinocyte, microbial, bone, and immune—of the periodontal microenvironment that leads to the pathogenesis of periodontitis are identified. These interactions are organized into 14 molecular systems involved in periodontitis.

Overall Conclusions. A molecular systems architecture is developed to provide a visual framework for comprehending the complexity of molecular interactions across the 8 cell types involved in periodontitis pathogenesis. The resulting architecture may be used for target identification and discovery of single and multicombination therapeutics to treat periodontitis more effectively.

Key Words. Periodontitis; gingival epithelial cells; immune cells; endothelial cells; inflammation; immune regulation; CytoSolve; systems biology.

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Introduction

Periodontitis is a multifactorial disease resulting from the disruption of host-microbial homeostasis. Periodontitis is an ancient disease that became prevalent after the domestication of plants and animals when *Porphyromonas gingivalis* and other periodontitis-associated bacteria became more common in the oral microbiome.[1,2] *P gingivalis* has been suggested to be a manipulator of the host response and an inducer of inflammation.[3,4] The polymicrobial synergy and dysbiosis model of periodontitis pathogenesis proposes that dysbiosis—a significant change in the relative abundance and transcriptomic activity in a bacterial community—promotes changes in host-microbial crosstalk, leading to the inflammation and bone loss[5-8] seen in periodontitis.

Periodontitis in adults is classified into 3 grades on the basis of a combination of 2 criteria, including the rates of bone loss and risk factors.[9] Grade A periodontitis is associated with a slower rate of bone loss (≈0.25% bone loss over 5 years) and heavy biofilm deposits in nondiabetic adults with no smoking history. Grade B periodontitis is associated with moderate loss of bone (0.25%-1.00% bone loss over 5 years), showing destruction because of biofilm deposits in adults with hemoglobin A_{1C} of less than 7% with a moderate smoking history (10 cigarettes per day). Grade C periodontitis is associated with a rapid rate of bone loss (> 1% bone loss over 5 years), showing severe destruction of bone because of biofilm deposits in adults with diabetes with a heavy smoking history (> 10 cigarettes per day).

Host-microbial homeostasis in the periodontium is maintained by a controlled inflammatory state.[10] Any disruption in the inflammatory status of the host brought about by environmental conditions or other predisposing factors can shift the balance toward dysbiosis, wherein microbes that were originally commensals become pathobionts.[11] Keystone pathogens can favor dysbiosis even when there are no predisposing factors. The inflammation caused by the dysbiotic microbiota depends on crosstalk signaling between complement and pattern recognition receptors, such as toll-like receptors, leading to the inflammatory destruction of soft and hard periodontal tissue.[1,12,13] This, in turn, provides breakdown products such as peptides as a nutrient to pathogens, thereby initiating a self-perpetuating cycle of further dysbiosis and tissue destruction.

Systems biology may provide an opportunity to advance interdisciplinary, systems-based research in periodontal disease. The focus of this effort is understanding periodontitis—a well-explored experimental area of research—from the systems biology perspective, which enables viewing complex diseases or biological functions as being composed of dynamic networks of biochemical reactions across multiple cellular systems.

Many subsystems beyond the biofilm and inflammatory response are involved in periodontal disease pathogenesis. Systems biology provides a valuable integrative approach to incorporate other subsystems involved in periodontitis. In 2021, Caetano et al[14] identified the heterogeneity of cellular components and receptor-ligand combinations occurring over the progression of periodontitis. Similarly, Williams et al[15] mapped the cellular environment in the oral mucosa with a specific focus on the role of neutrophil recruitment during disease progression. Studies such as these have focused on a systems approach to mapping cellular components, receptors, and ligands implicated in periodontitis.

The study aimed to develop a systems biology foundation to provide a comprehensive molecular systems architecture (ie, an interactome of the molecular interactions within and across the cells in the periodontal microenvironment). To develop such a foundation, this study focuses on *P gingivalis* to provide a molecular systems architecture of its interactions with the periodontal cellular microenvironment in periodontitis, which is, to the best of our knowledge, the first of its kind. The insights from this effort may provide the periodontitis research community with an integrative molecular systems understanding of the complexity of the biomolecular interactions involved in periodontitis pathogenesis and the foundation to expand the molecular systems architecture. The results of this investigation may also support the identification of potential targets for new therapeutic interventions.

Methods

The scientific literature was searched to identify articles that contain research on periodontitis, molecular pathways of periodontitis, cells in the oral cavity, interactions between oral cells and the oral microbiome, and the molecular pathways involved in the cellular crosstalk in the oral cavity. The CytoSolve systems biology tool (CytoSolve) used in this study enables the systematic bioinformatics literature review and scalable computational modeling of molecular pathways.[16-20] CytoSolve has been applied to diverse areas in systems biology, such as osteoarthritis,[21] neurovascular diseases,[22] and acute myeloid leukemia.[23] The protocol for setting up and using CytoSolve is provided in the Appendix.

Literature review process

Developing a systems architecture includes exploring the public repository of scientific articles. CytoSolve has been used to perform systematic reviews and support the curation and development of the molecular systems architecture of periodontitis pathogenesis. Search criteria are applied to optimize recall and precision to build a relevant repository of documents. In this process, the goal is to find all the potential documents (ie, high recall) and, from that list of potential documents, to discover those relevant (ie, high precision to the area of interest). The protocol for setting up and using CytoSolve is provided in the Appendix. The next

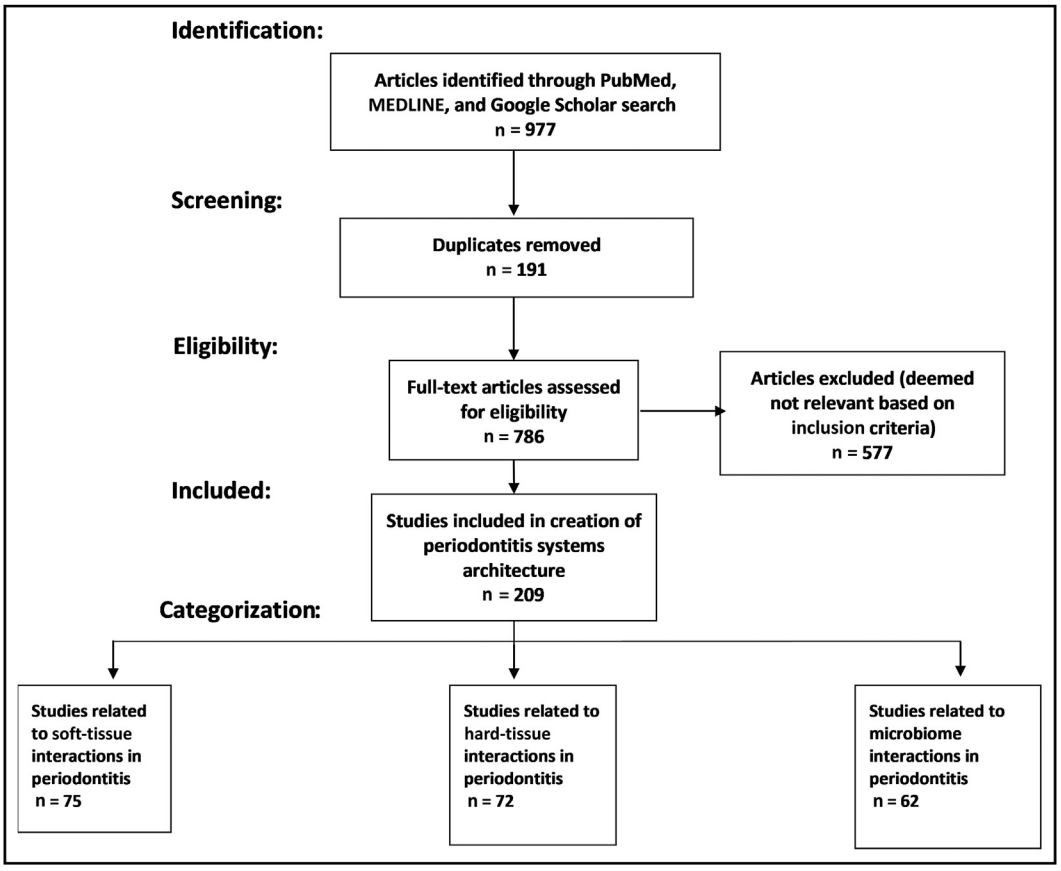

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram. A total of 977 articles were identified, 191 duplicates were removed, 786 articles were eligible for review, and 577 were removed as they were deemed not relevant, and 209 articles were included in the final analysis.

section details the inclusion and exclusion criteria used by CytoSolve.

Inclusion and exclusion criteria

The CytoSolve systematic bioinformatics literature review process and categorization are represented in Figure 1 as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[24]

The publication date for articles was restricted to January 1980 through April 2022 for studies using human and animal models. An article was deemed relevant only if the body of the article contained the key words in Table 1 with specific relation to periodontitis pathogenesis. In the screening process, unpublished literature was excluded as it had not undergone peer review to authenticate the results. Duplicate studies were removed from the initial set obtained after applying the inclusion criteria in Table 1.

The authors reviewed the titles and abstracts from the initial set of unique articles to determine whether an article contained information on molecular pathways related to the pathogenesis of periodontitis. Articles that did not contain information on molecular pathways related to the pathogenesis of periodontitis were excluded.

Table 1 MeSH* key words used for computer-based screening.

MeSH* Key Words
periodontitis AND oral microbiome
periodontitis AND IL-8† signaling
periodontitis AND LPS‡ signaling
periodontitis AND IL-1β§ signaling
periodontitis AND IL-6‖ signaling
periodontitis AND IL-4¶ signaling
periodontitis AND IL-10# signaling
periodontitis AND flagellin
periodontitis AND *P. gingivalis***
periodontitis AND endothelial Cell
periodontitis AND T cells
periodontitis AND epithelial cells
periodontitis AND ligament cells
periodontitis AND Th1†† cells
periodontitis AND Th17‡‡ cells
periodontitis AND macrophages
periodontitis AND neutrophils
periodontitis AND osteoblasts/osteoclast cells

*MeSH: Medical Subject Headings. † IL-8: Interleukin 8. ‡ LPS: Lipopolysaccharides. § IL-1β: Interleukin 1β. || IL-6: Interleukin 6. ¶ IL-4: Interleukin 4. # IL-10: Interleukin 10. ** *P. gingivalis: Porphyromonas gingivalis.* †† Th1: Helper T cell 1. ‡‡ Th17: Helper T cell 17.

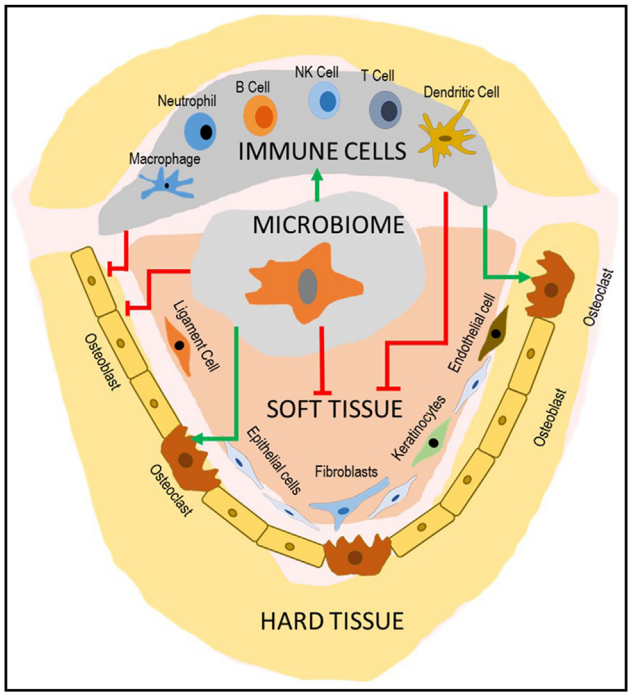

Figure 2 Periodontal cellular environment. The microbiome interacts with cells from the soft and hard tissues leading to the pathogenesis of periodontitis. An imbalance in the oral microbiome stimulates the activation of immune cells, mediating the degradation of soft and hard tissue.

The included list of final articles was divided into 3 categories: (1) studies related to molecular interactions in hard tissue leading to the pathogenesis of periodontitis, (2) studies related to molecular interactions in soft tissue leading to the pathogenesis of periodontitis, and (3) studies related to molecular interactions in the microbiome leading to the pathogenesis of periodontitis.

Journal articles from the final list were reviewed to identify molecular pathways involved in the pathogenesis of periodontitis. A molecular pathway involves chemical species (eg, proteins and messenger RNA) and their biochemical interactions in a particular compartment (eg, cell and organelle). The steps to acquire and represent molecular pathways diagrammatically from each article are as follows:

1. Identify and extract chemical species within a particular cell type and the associated cellular compartment in which the chemical species are present in each cell (eg, cytosol, mitochondria, and nucleus).
2. Identify the biochemical interactions within a particular cell type and the associated cellular compartment in which the chemical species are present in each cell.
3. Diagrammatically represent the molecular pathway, the network of chemical species identified in step 1, and the biochemical interactions identified in step 2.
4. Interconnect molecular pathways from step 3 in each cell type.

Results and Discussion

The microbial cells interact with the soft- and hard-tissue cells to effect gradual loss of these tissues leading to periodontitis. The signaling molecules that regulate these processes can originate from the bacterial cells or the proinflammatory immune cells; hence, their importance in developing a molecular systems architecture. A schematic of the periodontal cellular microenvironment is shown in Figure 2. The bioinformatics process for CytoSolve yields a schematic of the interactive signaling in the oral cavity, as shown in Figure 3A.

A detailed exposition of the critical interactive signaling mechanisms is provided below. This exposition provides the critical elements of the periodontitis molecular systems architecture.

Interactive crosstalk between microbial cells and immune cells

Periodontitis has been described as a microbial-shift disease because of a well-characterized change in the microorganisms (from mostly gram-positive to mostly gram-negative species) during the transition from periodontal health to disease. The microbial shifts found in diseased vs healthy sites are associated with changes in the clinical status of the tissue. Many bacterial species, including commensal and pathogenic bacteria, can change tissue homeostasis.[25] Dental plaque containing various microbial taxa can activate toll-like receptor 2 (TLR2), 4 (TLR4), or both and initiate a destructive inflammatory response, and the red-complex periopathogens such as *P gingivalis*, *Tannerella forsythia,* and *Treponema denticola* may also inhibit innate host defense functions.[25,26] The mechanisms by which periopathogens inhibit host defense functions are summarized below.

Interleukin 8 Signaling

A concentration gradient of interleukin 8 (IL-8) is essential in facilitating the migration of neutrophils and clearance of bacteria in the periodontal pockets, and commensal bacteria contribute to the induction of the IL-8 gradient. Pathogenic *P gingivalis* can inhibit the secretion of IL-8 from the gingival epithelium by many mechanisms. A phosphoserine phosphatase SerB from *P gingivalis* contributes to the inhibition.[27] *T denticola* can inhibit IL-8 function by inducing its degradation, mediated by its outer membrane protease dentilisin. *P gingivalis* possibly also contains proteins with strong proteolytic activity against IL-8. The inhibition of epithelial cell IL-8 responses by *P gingivalis* and the presence of a TLR4 antagonist may affect the interactions of the entire microbial community with the host. Once the innate immune status of the periodontal pocket is compromised by the reduction in IL-8 secretion, neutrophil transit may be disrupted, resulting in an increase in the number and types

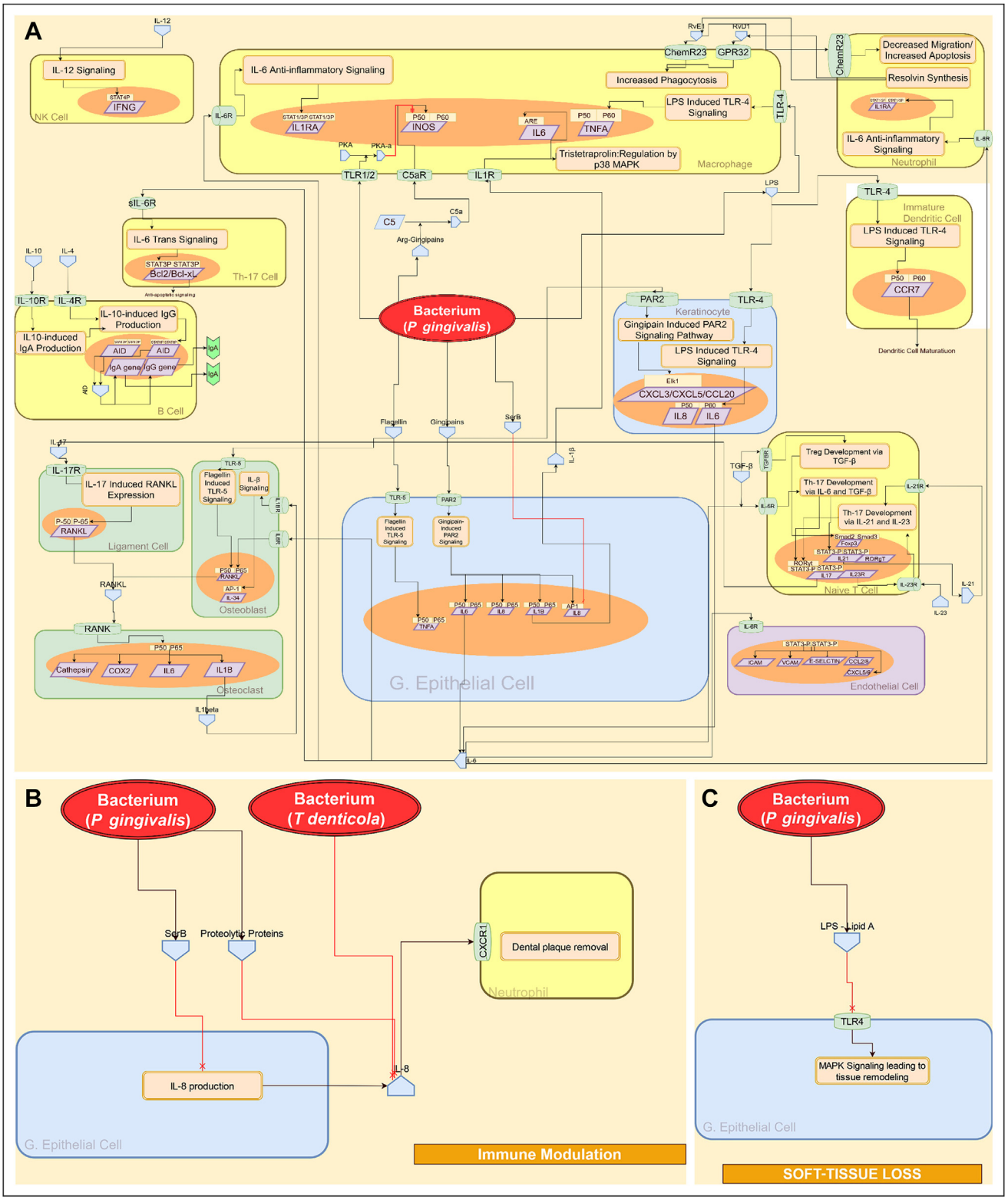

Figure 3 **A.** Schematics of interactive signaling among microbiome cells and soft- and hard-tissue cells derived from the CytoSolve bioinformatics process. **B.** Interactions among the microbiome, epithelial cells, and neutrophils leading to immune system modulation. **C.** Interactions between the microbiome and epithelial cells leading to soft-tissue loss. **D.** Interactions among the microbiome (represented by *P gingivalis*), immune cells (represented by macrophage), and hard tissue (represented by osteoclast, leading to hard-tissue loss). **E.** p38 mitogen-activated protein kinases signaling in neutrophils leads to immune activation. **F.** Interactions between the microbiome and epithelial cells leading to soft-tissue loss. **G.** Interactions between the microbiome and gingival fibroblasts leading to soft-tissue loss. **H.** Interactions among the microbiome, soft tissue, and hard tissue leading to bone loss. AID: Activation-induced cytidine deaminase. AP: Activator protein.

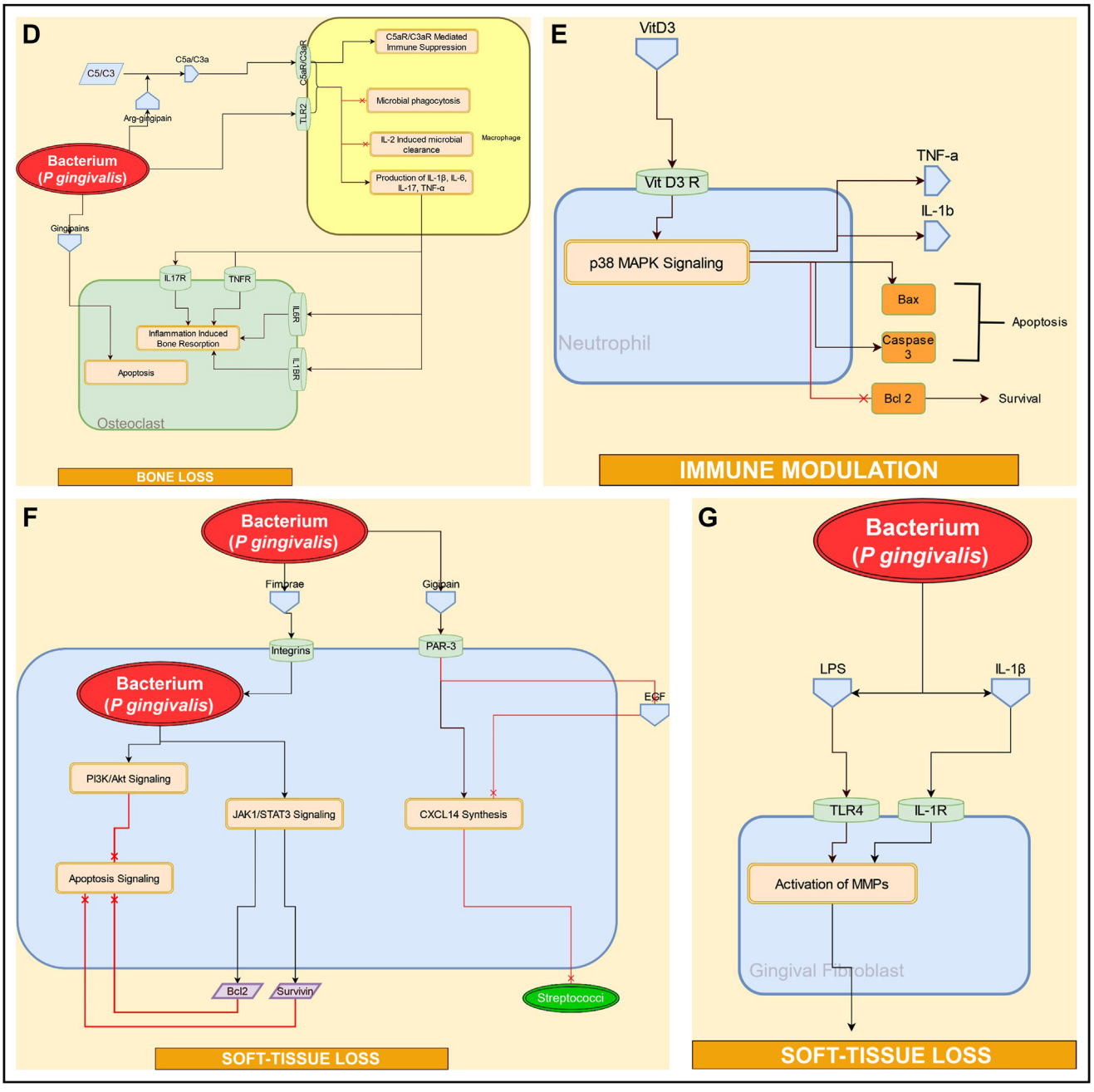

Figure 3 (Continued).

ARE: ATP-binding cassette repressor. Bax: Bcl-2 associated X-protein. Bcl: B-cell leukemia/lymphoma. Bcl-xL: B-cell leukemia/lymphoma-extra large. C: Component. CCL: Chemokine (C-C motif) ligand. CCR: Chemokine (C-C motif) receptor. ChemR: Chemerin receptor. COX2: Cyclooxygenase 2. CXCL: Chemokine (C-X-C motif) ligand. EGF: Epidermal growth factor. G. epithelial cell: Gingival epithelial cell. GPR: G-protein receptor. ICAM: Intercellular adhesion molecules. IFNG: Interferon genes. Ig: Immunoglobulin. IL: Interleukin. INOS: Inducible nitric oxide synthase. JAK: Janus kinase. LPS: Lipopolysaccharides. MAPK: Mitogen-activated protein kinase. MMP: Matrix metalloproteinase. mRNA: Messenger RNA. NK: Natural killer. p38 MAPK: p38 mitogen-activated protein kinase. P50: Protein 50 subunit. p53: Tumor protein P53. P60: Protein 60 subunit. PAR: Protease-activated receptor. *P gingivalis: Porphyromonas gingivalis*. PI3K: Phosphoinositide 3-kinase. PKA: Protein kinase A. RANK: Receptor activator of nuclear factor-κB. RANKL: Receptor activator of nuclear factor-κB ligand. RORgt: Retinoic acid receptor-related orphan receptor gamma. RvD: Resolvin D-series. RvE: Resolvin E-series. SerB: Serine phosphatase. STAT: Phosphorylated signal transducer and activator of transcription 1. *T denticola*: *Treponema denticola*. TGF: Transforming growth factor. TGFBR: Transforming growth factor beta receptor. Th17: Helper T cell 17. TLR: Toll-like receptor. TNF: Tumor necrosis factor. TNFR: Tumor necrosis factor receptor. VCAM: Vascular cell adhesion molecule. VitD3: Vitamin D3. Vit D3 R: Vitamin D3 receptor.

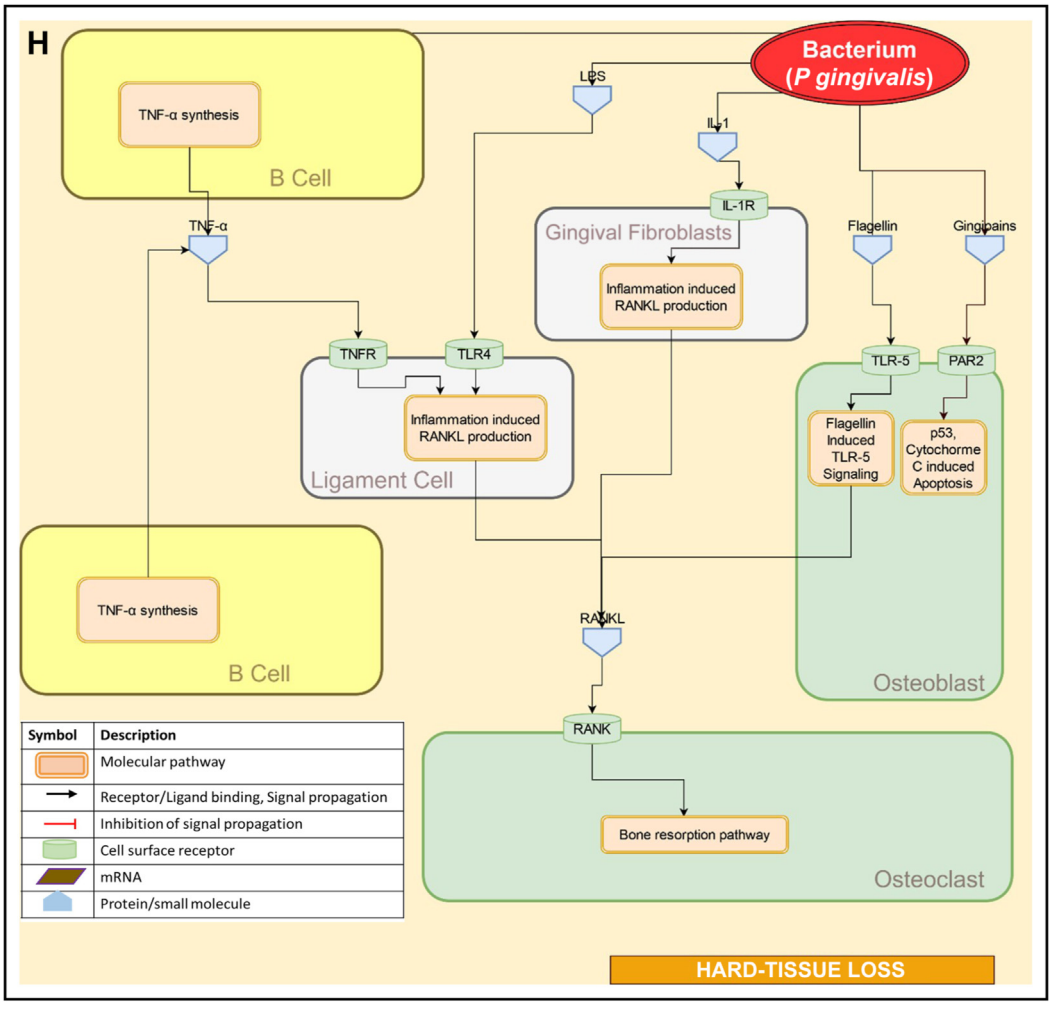

Figure 3 (Continued).

of bacteria in the pocket.[25] The schematics of these interactions are shown in Figure 3B.

Lipid Raft Signaling

P gingivalis can manipulate host regulatory mechanisms by inducing a crosstalk between TLR2 and CXC chemokine receptor 4 after their recruitment to lipid rafts in response to *P gingivalis* fimbriae. This reduces the host protective response against the pathogen.[28] Lipid A is part of the lipopolysaccharide on the outer membrane of gram-negative bacteria that can function as an antagonist of TLR4.[29] The structure of *P gingivalis* lipid A is heterogeneous with a 5-acyl monophosphate structure, which is a weak TLR4 agonist, whereas a 4-acyl monophosphate structure is a TLR4 antagonist. The expression of both the TLR4 agonist and antagonist lipid A structures in *P gingivalis* are regulated by the hemin concentration in the growth medium or microenvironment, most likely through lipid A phosphatases. Secreted TLR4 antagonists can interrupt epidermal growth factor–dependent signaling pathways involved in the remodeling of the periodontal tissue at the level of extracellular signal-regulated kinase 1, extracellular signal-regulated kinase 2, p38 mitogen-activated protein kinase (MAPK), and cyclic adenosine monophosphate-response element binding protein proteins, which are components of the MAPK signaling cascade.[30-32] The schematics of these interactions are represented in Figure 3C.

Complement System in Periodontitis

The complement system is strongly involved in the dysbiotic transformation of periodontal microbiota and the destructive inflammatory response characteristic of periodontitis. Clinically, gingival crevicular fluid samples from patients with periodontitis contain higher levels of activated complement fragments than samples from healthy people.[33,34] At the molecular level, the complement component 5a (C5a) receptor (C5aR/CD88) is a target of immune subversion by *P gingivalis* leading to the dysbiotic transformation of the microbiota. C5aR engages in crosstalk with TLR2 to disarm the protective TLR2-MyD88 pathway in the host, also dissociating it from the sTLR2–MyD88 adaptorlike (Mal)–phosphoinositide 3-kinase pathway that

prevents phagocytosis of *P gingivalis*. This C5aR-TLR2 subversive crosstalk undermines the killing function of macrophages and neutrophils.[35,36]

The *P gingivalis*-induced C5aR-TLR2 crosstalk also inhibits TLR2-induced interleukin IL-12p70 (IL-12), but it upregulates inflammatory and bone resorptive cytokines such as interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α). This inhibition enables *P gingivalis* to escape IL-12–dependent immune clearance in vivo and to cause inflammatory bone loss.[37] The significance of C5aR in immune evasion by *P gingivalis*, contributing to periodontitis, makes it an attractive target for therapeutic intervention. C5aR inhibitors may have the potential to treat other infections associated with *P gingivalis*, such as aspiration pneumonia and atherosclerosis.[37]

Despite excessive complement activation in periodontal conditions, periodontal bacteria possess mechanisms to evade complement-mediated killing.[34] The C5aR-TLR2 crosstalk leads to a dysbiotic transformation of the periodontal bacteria, resulting in C3-dependent inflammatory bone loss.[34] In addition to their action on C5, Arg-gingipains can also degrade C3, thereby inhibiting complement activation regardless of the initiation pathway involved. During the initial stages of infection, when *P gingivalis* is present in low numbers and when the concentration of gingipains is low, the complement protein C1 is activated but is unlikely to kill the bacteria. However, the resulting local inflammatory response might provide the bacteria with nutrients such as gingival crevicular fluid-derived hemin, an iron source.[37] The complement system interactions in periodontitis are illustrated in Figure 3D.

p38 Signaling Induced Apoptosis of Neutrophils

Neutrophils constitute the front line of host defense in the innate immune system and have key positions in inflammatory reactions.[38] However, continual activation of neutrophils leads to a chronic proinflammatory environment that results in periodontitis progression.[39] A 2017 study showed that periodontitis was more likely to occur in adults with uncontrolled type 2 diabetes mellitus.[40] In periodontitis patients with type 2 diabetes mellitus, treatment with 1,25-dihydroxy vitamin D3 (1,25VitD3) enhanced the apoptosis of neutrophils, indicating that the 1,25VitD3 levels may play a significant role in modulating apoptosis.[41] Mechanistically, 1,25VitD3 activates the p38 MAPK pathway, which regulates neutrophil apoptosis. Treatment of neutrophils with 1,25VitD3 induced the expression of proapoptotic markers such as caspase-3 and Bax while downregulating antiapoptotic markers, including Bcl2[41] resulting in upregulation of neutrophil apoptosis. The schematics of p38 MAPK signaling in neutrophils are shown in Figure 3E.

Interactive Crosstalk Signaling Between P gingivalis and Soft-Tissue Cells

The invasion of epithelial cells by bacteria begins with the attachment of bacteria to the epithelial cell membrane. This may involve many effector molecules. The predominant adhesins mediating the attachment of *P gingivalis* are fimbriae, composed of FimA, FimB, and FimC subunits, of which FimA directly engages integrins on the surface of the gingival epithelium.[42] This interaction initiates an integrin-associated signaling cascade that triggers bacterial internalization that involves the recruitment of focal adhesion adaptors, signaling proteins such as paxillin and focal adhesion kinase to sites of *P gingivalis* attachment and the subsequent entry of *P gingivalis*.[43]

The internalization of *P gingivalis* accelerates the progression of the S-phase and upregulates the proliferation of gingival epithelial cells.[44] In addition, the bacterial interactions also upregulate antiapoptotic signaling in the epithelial cells by activation of phosphoinositide 3-kinase/Akt and Janus kinase-1/signal transducer and activator of transcription protein-3 pathways, leading to the expression of antiapoptotic genes, including Bcl-2 and survivin, and inhibition of cytochrome c and caspase 3/9.[45,46] The increased proliferation of epithelial cells may allow the bacteria a larger number of cell to infect and replicate in without increasing bacterial burden in an individual cell.[47] The interactions between bacteria and epithelial cells are illustrated in Figure 3F.

CXCL14 acts as a bactericidal agent against streptococci, part of the healthy oral microbiome. *P gingivalis* dysregulates CXCL14 expression and may promote dysbiosis and the development of periodontitis.[48] As a response to gingipains from *P gingivalis*, a chronic inflammatory environment is created in the oral epithelial cells leading to upregulation of CXCL14 via protease-activated receptor-3.[48] Under normal circumstances, CXCL14 expression is found to be transcriptionally repressed by epidermal growth factor–induced activation of the mitogen-activated protein kinase kinase–extracellular signal-regulated kinase 1/extracellular signal-regulated kinase 2 pathway. Under dysbiosis, *P gingivalis* overcomes this repression via the gingipain protease-mediated degradation of epidermal growth factor.[49] Thus, *P gingivalis* induces dysbiosis in the oral cavity by suppressing the healthy oral microbiome via CXCL14. *P gingivalis* also induces pyroptosis (cell death that is triggered by proinflammatory signals and associated with inflammation) of gingival fibroblasts via lipopolysaccharide signaling, leading to soft-tissue loss.[50]

Bacterial components such as lipopolysaccharides and cytokines activate macrophages to produce cytokines such as IL-1β and TNF-α.[51] These cytokines activate the fibroblasts that reside in the periodontal tissues to produce the matrix metalloproteinases (MMPs), a plasminogen activator, which can activate plasmin. Plasmin, in turn, can activate other types of MMPs that can be inhibited by tissue inhibitors of metalloproteinases.[52] The increased and prolonged bacteria-induced MMPs promote enhanced degradation of collagen—a primary component of the periodontal extracellular matrix. MMP-8 and MMP-9 are also released from the polymorphonuclear leukocytes and contribute

substantially to soft-tissue loss initiated by the host response, as observed in patients with chronic periodontitis.[48] The interactions between bacteria and gingival fibroblast leading to activation of MMPs are shown in Figure 3G.

Interactive Signaling Between the Microbiome and Hard-Tissue Cells

Bone is a dynamic tissue that undergoes constant renewal in response to mechanical, nutritional, inflammatory, and hormonal stimuli. Osteoblasts are cells with a primary role in bone formation and express a range of chemokines, prostaglandins, and growth factors. Osteoclasts are highly specialized motile bone resorptive cells derived from hematopoietic stem cells of the monocyte/macrophage lineage in response to factors such as macrophage colony-stimulating factor-1, receptor activator of nuclear factor-κB ligand (RANKL), and numerous cytokines.[53-55] A balance between the processes of bone resorption by osteoclasts and bone formation by osteoblasts is required for the proper maintenance of bone homeostasis.

Various factors regulate bone resorption by osteoclasts in periodontitis, including hormones, growth factors, and cytokines. Proinflammatory cytokines, including IL-1β, interleukin 6 (IL-6), and TNF-α, stimulate osteoclastic resorption via RANKL, whereas interleukin 10 and transforming growth factor beta (TGF-β) are potent inhibitors of osteoclast activity.[56,57] IL-1β has been shown to promote the inhibition of reparative bone formation by osteoblasts in periodontitis.[58,59] Thus, although bone turnover is stimulated, unlike in a healthy periodontium, new bone formation to repair that which is lost does not occur in periodontitis. RANKL and osteoprotegerin (OPG) are members of the TNF superfamily whose balance regulates osteoclastogenesis.[60] OPG is a negative regulator and RANKL is a positive regulator of osteoclastogenesis through interaction with appropriate receptors on monocyte and macrophage cell lineage cells. RANKL-mediated osteoclastogenesis plays a pivotal role in inflammatory bone resorption, and its expression is increased in periodontitis.[60] The periodontopathic bacteria *Aggregatibacter actinomycetemcomitans* and *P gingivalis* have unique mechanisms to induce RANKL in osteoblasts and gingival fibroblasts. When stimulated with lipopolysaccharide and interleukin 1, these cells express RANKL.[61] In 2015, it was shown that RANKL is upregulated, whereas OPG is downregulated in periodontitis compared with periodontal health, resulting in an increased RANKL/OPG ratio leading to bone resorption.[62]

Bone resorption in periodontitis occurs via the concerted action of osteoclast-stimulating or -inhibiting cytokines in the inflamed tissue, which are produced by both immune, such as B and T lymphocytes, and resident cells, including gingival fibroblasts and periodontal ligament cells.[63,64] During homeostatic bone remodeling, osteoclastic activity is triggered via RANKL bound on the surface of osteoblasts, which activates the receptor activator of nuclear factor-κB receptor on the surface of osteoclasts. RANKL can exist in membrane-bound or soluble form (sRANKL).[65] sRANKL in the gingival crevicular fluid is highly correlated with the severity of periodontitis. sRANKL can be formed from membrane-bound RANKL by the action of enzymes such as the TNF-α converting enzyme, which is produced by T cells challenged with the periodontal pathogen *P gingivalis*.[66] sRANKL and TNF-α released from lymphocytes by TNF-α converting enzyme–mediated cleavage can activate osteoclast precursors to induce osteoclastogenesis from the alveolar bone surface in the periodontitis lesion.[67]

Molecules produced by periodontal pathogens can upregulate RANKL expression in gingival fibroblasts. Cytolethal-distending toxin produced by *A actinomycetemcomitans* can induce in vitro RANKL expression from human gingival fibroblasts.[68] Arg-gingipains produced by *P gingivalis* were also found to upregulate the RANKL/OPG expression ratio in gingival fibroblasts.[64] In addition to stimulating osteoclastogenesis, gingipains are also implicated in the apoptosis of osteoblasts,[69] via upregulation of active p53, caspase 3, and translocation of Bid3 into mitochondria leading to cytochrome c release.[70] The interactions among immune cells, bone cells, and the bacteria leading to bone loss are shown in Figure 3H.

Conclusions

This CytoSolve systematic bioinformatics review identified critical molecular systems components of periodontitis pathogenesis. The organization of these components into a molecular systems architecture is presented in Figure 4. The bottom layer of Figure 4 represents cellular components of the oral microenvironment: gingival epithelial cells, fibroblasts, periodontal ligament cells, endothelial cells, microbial cells, bone cells, and immune cells. The middle layer of Figure 4 represents the key molecular interactions implicated in the pathogenesis of periodontitis: flagellin-induced toll-like receptor-5 (TLR5) inflammatory signaling in gingival epithelial cells, keratinocytes, and bone cells; protease-activated receptor 2 signaling in gingival epithelial cells, interleukin 17 signaling in ligament cells; IL-6 signaling in endothelial cells and immune cells; flagellin and gingipains signaling in bacterial cells; RANKL signaling in fibroblasts and bone cells; and signaling by interleukin 23, IL-8, interleukin 21, TLR-4, C5/C3 complement system, TGF-β, and IL-12 in immune cells. The top layer of Figure 4 represents the biological processes implicated in the pathogenesis of periodontitis: soft-tissue loss, bone loss, and immune modulation.

The molecular systems architecture in Figure 4 provides a consolidated guide to understanding the overall pathogenesis of periodontitis. Interactions among the 8 cell types in the bottom layer give rise to 14 molecular systems in the middle layer. Of these 14 molecular systems components, 7

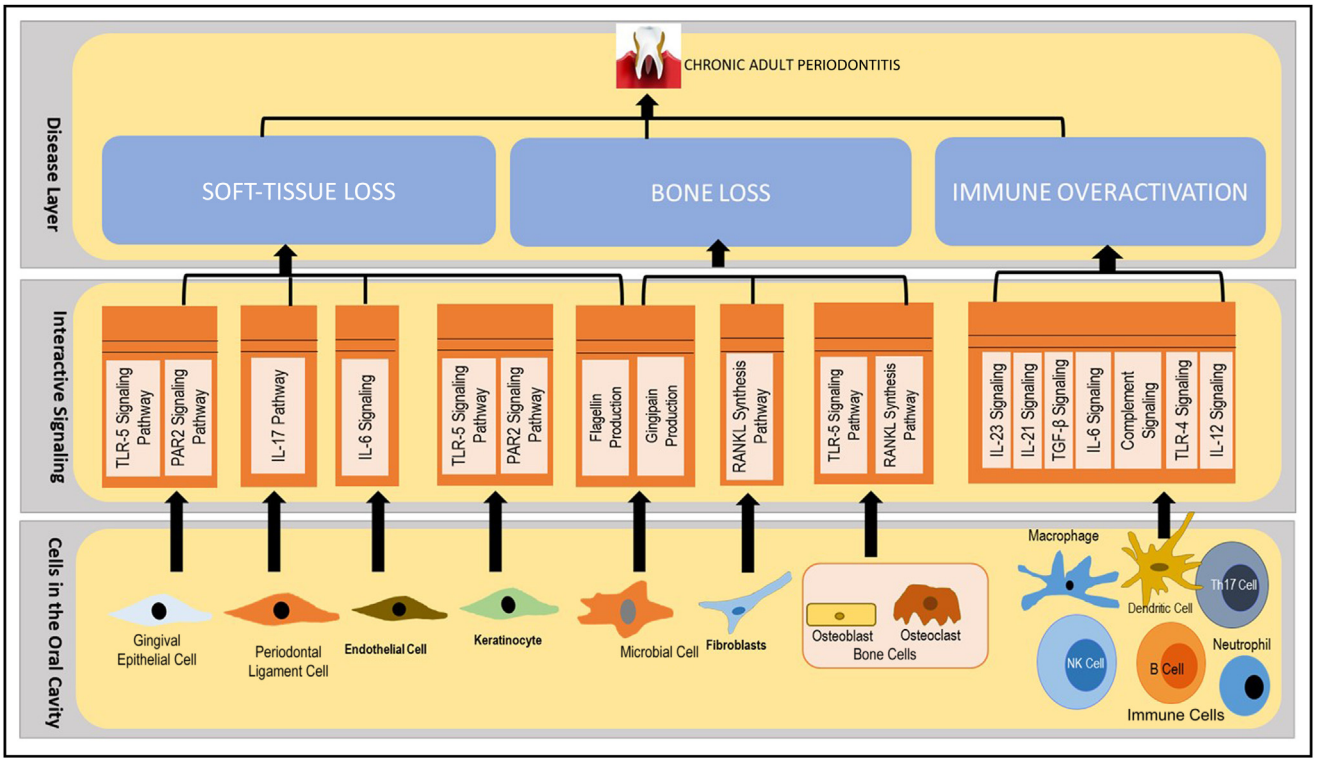

Figure 4 Molecular systems architecture of interactive signaling in periodontitis. In the 3-layered architecture, the bottom layer consists of cellular factors involved in the pathogenesis of periodontitis. The middle layer consists of the interactions within and among the cellular components. The top layer represents the biological processes resulting from the interactions in the oral microenvironment. IL: Interleukin. NK: Natural killer. PAR: Protease-activated receptor. RANKL: Receptor activator of nuclear factor-κB ligand. TGF: Transforming growth factor. Th17: Helper T cell 17. TLR: Toll-like receptor.

(TLR5, PAR2, TGF-β, interleukin 17, IL-6, flagellin, and gingipains) contribute to periodontitis pathogenesis by promoting soft-tissue loss. Four molecular systems components, signaling by TLR5, RANKL, flagellin, and gingipains, contribute to periodontitis pathogenesis by promoting bone loss and preventing bone repair. Seven molecular systems components: singling by interleukin 23, IL-8, interleukin 21, TLR-4, complement system, TGF-β, and IL-12, contribute to periodontitis pathogenesis by modulating the immune system. The integrated processes of soft-tissue loss, bone loss, and immune modulation, driven by the molecular subsystems in their respective cellular interactions, give rise to periodontitis.

The architecture also may offer a vehicle for new insights and discovery. For example, we have identified several targets across different cell types in the microenvironment that can potentially be used to develop therapeutic interventions to inhibit the inflammatory processes and bone and soft-tissue loss, listed in Table 2.

In summary, as shown in Figure 4, this molecular systems architecture visually represents the systems biology of periodontitis on the basis of the current science reviewed

Table 2 Summary of potential therapeutic molecular targets.*

Physiological Effect	Cell Type	Potential Target
Inhibition of inflammatory microenvironment	Gingival epithelial cells, immune cells	Interleukin 1β, interleukin 8, tumor necrosis factor-α, interleukin 6, complement 5a
Inhibition of bone loss	Periodontal ligament cells, osteoblast or osteoclast	Receptor activator of nuclear factor kappa-B ligand, interleukin 6 receptor, receptor activator of nuclear factor kappa-B
Inhibition of soft-tissue loss	Gingival epithelial cells	Toll-like receptor 5, proteinase-activated receptor 2

*The targets are categorized according to physiological effects. Five molecular targets were identified in suppressing inflammation across gingival epithelial cells and immune cells. Three targets were identified across periodontal ligament cells and osteoblasts or osteoclasts. Two targets were identified in gingival epithelial cells.

and curated. The architecture provides a framework for scientific collaboration and instantiation of future knowledge based on new science and feedback from the periodontitis community.

Limitations and future directions

Limitations

In this review, per the aims of this study, we have focused on *P gingivalis* as a representative species of the pathogenic bacteria because it is a well-studied organism in the periodontitis literature.[71-73] The Medical Subject Headings key words and key word phrases, in Table 1, constrained the literature to this representative species. The framework created from this effort may be used to expand to other bacterial species, such as *T denticola* and *T forsythia* and their interactions with the periodontal microenvironment, which is a subject of ongoing and future work.

Future Directions

The molecular systems architecture developed in this review provides a blueprint for understanding the complex interactions occurring in the periodontitis microenvironment. This understanding will enable the identification of targets in the interactive signaling pathways that may be used to develop novel combination therapies and synthetic approaches that may be more effective than the current therapeutic options and potentially mitigate undesirable side effects.

The molecular systems architecture provides a versatile tool for identifying how targeting a particular mechanism in an oral cell type can have either a positive or negative cascading effect on the rest of the oral microenvironment, hence providing a much better drug development paradigm that can minimize side effects and maximize the efficacy of treatment. This molecular systems architecture can be further enhanced to include additional factors that may predispose people to or protect them from disease, such as host genetics (ie, single nucleotide polymorphisms, and methylation), epigenetics, environmental factors, and systemic health status, because all these factors may play a significant role in host-microbiome responses. This architecture may also be converted to an open science interactive web-based tool to enable ongoing collaborative development from the periodontitis research community. Such efforts have been made successfully in neurovascular diseases, human knee osteoarthritis,[21] and acute myeloid leukemia.[23]

Mechanistic in silico modeling is emerging as a valuable preclinical drug discovery tool. The molecular systems architecture we present provide a starting point for such mechanistic in silico modeling efforts. The computational capabilities of CytoSolve can be used to create an integrative computational model for the oral microenvironment. The resulting in silico oral microenvironment model can then be used as a testing and validation platform to identify new targets and novel combination therapies.

Clinical relevance

A molecular systems representation of the host-microbial interactions in the periodontium is needed to understand the complexity of the pathogenesis of periodontitis. In this review, a molecular systems architecture of periodontitis has been developed to represent biological processes, including soft-tissue and bone loss and immune modulation within and across the various cellular components of the periodontium. The resultant periodontitis molecular systems architecture allows for identifying potential targets across many periodontal cell types to develop new therapeutic interventions that may inhibit these inflammatory processes and reduce or prevent bone and soft-tissue destruction.

Disclosures

Drs Ayyadurai and Deonikar are employees of CytoSolve. None of the other authors reported any disclosures.

Supplemental Data

Supplemental data related to this article can be found at: https://doi.org/10.1016/j.jfscie.2023.100026.

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