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New insights into the emerging role of oral spirochaetes in periodontal disease

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Abstract

Spirochaetes are prominent in the polymicrobial infections that cause periodontal diseases. Periodontitis is a chronic inflammatory condition of the periodontium, characterized by proinflammatory soft tissue damage and alveolar bone loss. *Treponema denticola* is the most well-understood oral spirochaete, expressing a wealth of virulence factors that mediate tissue penetration and destruction as well as evasion of host immune responses. This review focuses on emerging knowledge of virulence mechanisms of *Treponema denticola* as well as mechanisms of other less-studied oral treponemes.

Keywords: Oral, pathogenesis, periodontitis, review, spirochaete, *Treponema*, virulence
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Introduction

Periodontitis is characterized by chronic inflammation, alveolar bone loss and destruction of the gingival and periodontal ligament attachment to teeth, coincident with a shift in the microbial population in the gingival pocket. Spirochaetes comprise up to 50% of the polymicrobial population in subgingival plaque in periodontitis, and <1% in health [1]. Spirochaetes are divided into three families: the *Spirochaetaceae*, *Leptospiraceae* and *Brachyspiraceae* [2]. Only phylotypes of the genus *Treponema*, a member of the *Spirochaetaceae* family, have been found in the mouth [3]. Ten species of *Treponema* (*Treponema denticola* [4], *Treponema pectinovorum* [5], *Treponema socranskii* [6], *Treponema vincentii* [7], *Treponema lecithinolyticum* [8], *Treponema maltophilum* [9], *Treponema medium* [10], *Treponema parvum* [11], *Treponema putidum* [12] and *Treponema amylovorum* [13]) have been cultivated from the oral cavity, whereas over 70% of *Treponema* phylotypes remain uncultivatable, their characterization being limited to genetic identification [3,14]. *T. denticola* is well characterized in terms of its pathogenic mechanisms and association with periodontitis. *T. denticola* expresses a variety of factors that allow for its survival, host tissue penetration and immune evasion. *Treponema* species in addition to *T. denticola* have also been identified in various forms of disease and at differing pocket depths [15], raising a need for greater understanding of their potential virulence.

Advances in genome sequencing have furthered our understanding of the pathogenicity of oral spirochaetes. The genome of *T. denticola* ATTC 35405 has been annotated [16], and annotation of *T. vincentii* ATCC 35580 (Human microbiome project, Venter Institute) and *T. lecithinolyticum* OMZ 684T (Human Oral Microbiome Database) is underway. Genetic manipulation, including directed gene mutagenesis and plasmid transformation, of *T. denticola* has become more routine [17–19]. The development of a transposon system for *T. denticola* provides new opportunities for whole genome mutagenesis and investigation [20].

A number of recent reviews have described the virulence factors of *T. denticola* in detail [21–24] (Table 1). This review focuses on emerging knowledge of the pathogenic factors of *T. denticola* and factors established in other oral treponemes, selected for their novelty and likelihood of leading to major advances.

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TABLE 1. Pathogenic factors of oral spirochaetes

Pathogenic factor	Activity	References
Adhesins		
Major outer sheath protein (Msp)	Binding to fibronectin, laminin, collagen types I and IV, hyaluronic acid	[67,71,137]
	Co-aggregation with *Porphyromonas gingivalis* and *Fusobacterium nucleatum*	[51]
Oligopeptide transporter unit (OppA)	Binding to soluble fibronectin and plasminogen; not immobilized forms or epithelial cells	[68]
Dentilisin (PrtP, CTLP)	Adherence to fibrinogen	[66]
	Ligand for co-aggregation with *P. gingivalis* fimbriae	[49]
Fibronectin-binding protein (Fbp, 52 kDa)	Adherence to soluble and immobilized fibronectin	[59]
Leucine-rich repeat (LrrA)	Adherence/penetration of epithelial cells.	[50]
	Ligand for co-aggregation with *Tannerella forsythia*	
FHL-1-binding protein B (FhbB)	Adherence to factor H-like protein 1	[138]
Collagen-binding protein	Binding to collagen types I, IV and V	[139]
Td92	Binding to epithelial cells	[119]
M23 domain fibronectin-binding family of proteins	Binding to matrix and plasma fibronectin	[69]
Proteases/peptidases	Substrates	
Dentilisin	Transferrin, laminin, collagen, fibronectin, IgG, fibrinogen, α_1-antitrypsin, complement C3, IL-8, IL-6, TNF-α, intercellular adhesions, bradykinin, substance P, angiotensin I	[79,80,82,83,99,140]
Trypsin-like protease (OpdB)	*N*-α-benzoyl-DL-arginine-2-naphthylamide (BANA)	[85]
	Ester, amide and peptide bonds involving arginine and lysine	[87]
Dentipain (cysteine protease)	Insulin β-chain	[91]
Proline iminopeptidase	Dipeptides: Pro-Arg, Pro-Lys, Pro-Gln, Pro-Asn, and Pro-Ala	[86]
Endopeptidases	Bradykinin, collagenase substrates	[88]
	Substance P, neurotensin, angiotensins, oxytocin, vasopressin, and human endothelin fragment 22–38	[141]
Cytoxicity		
Msp	Pore formation in cell membranes	[70,75]
	Lysis of epithelial cells, erythocytes	[92]
	Cytoskeleton disruption, impaired host cell migration, disruption of calcium signalling	[93,97,128,129]
Dentilisin	Lysis of epithelial cells, cytoskeleton disruption	[92,96]
Cytalysin	Haemolysis	[37,142]
Motility		
Periplasmic flagella	Directed movement, cell invasion	[143,144]
Chemotaxis system	Environmental sensing and response, cell/tissue invasion	[101]
Immune activation		
Msp	TNF-α production through TLR2–MyD88 in macrophages	[115]
MspTL	ICAM-1, IL-6, IL-1β, IL-8, IFN-β, COX-2, RANTES and PGE_2 production in monocytes and PDL cells	[113,114,122]
MspA	ICAM-1, IL-6 and IL-8 production in monocytes and PDL cells	[114]
Td92	IL1-β, TNF-α, IL-6, COX-2 and PGE_2 production in monocytes and PDL cells	[119]
LOS	TLR4–MyD88 activation in macrophages.	[115]
	IL-6, IL-8, MCP-1, nitric oxide and PGE_2 production in fibroblasts	[124]
Glycolipids	TLR2–MyD88 activation	[116]
Peptidoglycan	IL1-β, IL-6, IL-8, TNF-α, RANTES and PGE_2 production in macrophage-like cells	[145]
Lipoprotein	Nitric oxide, TNF-α and IL-1 production in macrophages	[117]
Immune evasion		
Resistance to defensins	Inhibit human β-defensins 1, 2 and 3 through TLR2	[146–148]
TLR inhibition (immune tolerance)	Glycolipids or phospholipids inhibit TLR activation with CD14 and LPS-binding protein of host cells	[121,125,126,149]
	Msp and LOS mediate macrophage tolerance through TLR4 inhibition	[115]
Msp	Inhibits neutrophil polarization and chemotaxis through Rac1 inhibition	[128]
	Perturbs actin assembly, calcium transients and phagocytosis in neutrophils	[93,129]
Osteoclastogenesis		
Td92	Osteoclast formation in clavaria–bone marrow cell co-culture	[150]
	Increased production of RANKL and PGE_2, decreased OPG production in osteoblasts	
LOS	Osteoclast formation in clavaria–bone marrow cell co-culture	[151]
	Increased expression of RANKL and PGE_2, decreased OPG production in osteoblasts	
Mobile DNA elements		
Bacteriophage (φtd1)	Temperate bacteriophage	[45]
	Genetic transfer, survival?	
Transposases	IPR010106 family	[45]
	Genetic transfer, regulation?	
Miscellaneous		
Toxin–antitoxin system	33 predicted systems	[45]
	Programmed cell death? Biofilm persistence?	
Two-component systems	AtcSR system	[27]
	Survival? Virulence?	
	Hpk2–Rrp2 system	[28]
	Oxygen sensing? Survival?	
Metal transport/regulation		
Haemin-binding protein (HbpA, HbpB)	Haemin binding, iron acquisition	[34,152]
Lactoferrin-binding protein	Iron acquisition	[36]
Transport-related operon (TroABCD)	Manganese and iron transport, manganese-dependent and iron-dependent transcriptional regulator (TroR)	[39]
Host protease modulation		
Td92	MMP-9 production in monocytes	[119]
LOS	MMP-3, MMP-8, MMP-9, MMP-10, MMP-13 and MMP-14 gene transcription in osteoblasts	[151]
	MMP-3 production in fibroblasts	[124]
Peptidoglycan	MMP-9 production in macrophage-like cells	[145]
	MMP-9 production in neutrophils	[153]
Msp	MMP-9, cathepsin G, elastase and MMP-8 production in neutrophils	[153]
Dentilisin	MMP-2 activation	[83]

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TABLE 1. (Continued)

Pathogenic factor	Activity	References
Host cell signalling		
LOS	Fos, MKK1, MKK2, MKK3/6, NF-κB p50 and NF-κB p65 phosphorylation in fibroblasts	[124]
Peptidoglycan	ERK1/2, GRK2 and Lyn phosphorylation in macrophage-like cells	[145]
Msp	ERK1/2 and p38 phosphorylation in fibroblasts. Additional stress kinases activated in phosphokinase screening assays	[154]
	Rac1, RhoA and Ras activation in fibroblasts	(M. B. Visser, R. P. Ellen, unpublished data)
	Rac1 inhibition in neutrophils	[128]
MspTL	STAT-1 phosphorylation in monocytes	[113]

COX, cyclooxygenase; ICAM, intercellular adhesion molecule 1; IFN, interferon; IL, interleukin; LOS, lipooligosaccharide; LPS, lipopolysaccharide; MCP, monocyte chemotactic protein; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappaB; OPG, osteoprotegerin; PDL, periodontal ligament; PGE, prostaglandin E; TLR, Toll-like receptor; TNF, tumour necrosis factor.

Environmental Sensing and Adaptation by *T. denticola*

The periodontal pocket undergoes dramatic environmental changes during disease pathogenesis [25]. How oral spirochaetes sense and respond to their changing extracellular environment is relatively unknown, although two-component systems (TCSs) are key signal transduction elements involved in adaptation. TCSs consist of a sensor histidine kinase and a response regulator that influences gene transcription and cellular activity [26]. *T. denticola* genome annotation has revealed eight putative histidine kinases and nine response regulators [16]. The AtcSR and Hpk2–Rrp2 TCSs have recently been characterized in *T. denticola*. These were confirmed to encode functional systems, with expression in a growth-dependent manner [27,28]. The AtcR regulator sequence contains a LytTR domain [27], which affects virulence factors such as polysaccharide synthesis, fimbriae, toxin production and quorum sensing in other microorganisms [29].

The Hpk2 regulator evidently contains a PAS-haeme-binding domain, which functions in oxygen sensing [28], suggesting involvement of this TCS in treponemal responses to changing oxygen tension in the periodontal pocket. The Hpk2–Rrp2 TCS is part of a larger operon, which includes genes involved in peptidoglycan synthesis, DNA replication and translation, possibly allowing *T. denticola* to outgrow other microorganisms in the diseased periodontal pocket [28].

Recently, *T. denticola* genome profiles in response to environmental stresses encountered in the periodontal pocket, including heat shock, osmotic downshift, oxygen exposure and blood exposure, were examined [30]. Although each condition identified a specific set of genes that changed upon exposure, a set of 'core stress response' genes induced across all conditions were also identified. These included genes encoding chaperones and proteases, consistent with general cellular stress responses, along with a predicted σ^{70}-factor (TDE0937), which may be a global regulator of the stress response.

Blood exposure does not appear to activate a severe stress response, consistent with the fact that *T. denticola* resides in an environment that is prone to bleeding and has been implicated in systemic infections. However, a specific set of genes was activated, including transcriptional regulator and transport genes, probably representing genes relevant to infection and survival [30]. Also, transcription of treponeme surface antigens able to initiate an immune response in humans [31] was downregulated following blood exposure, representing a possible immune evasion strategy [30].

Like most bacteria, oral spirochaetes require essential elements such as iron, zinc and manganese for survival. Although these elements are often not freely available in the human host, fluctuations may occur because of bleeding and increased gingival crevicular fluid flow. *T. denticola* is known to possess orthologues of many metal-dependent enzymes and at least eight metal uptake pathways [16,32]. Both lactoferrin-binding and haemin-binding proteins, involved in iron acquisition, have been characterized in *T. denticola* [33–36]. Also, a haemolysin (cytalysin) has been reported to lyse erythrocytes and haemoxidize haemoglobin [37] as well as acting as a cysteine desulfhydrase to produce pyruvate as an energy source, and the toxic metabolites ammonia and hydrogen sulphide [38]. A troABCD operon, encoding a zinc and manganese transport system, has also been characterized [39]. The iron-dependent and manganese-dependent transcriptional repressor, TroR, is also present, acting to negatively regulate the Tro operon. It probably plays an important role in manganese and iron homeostasis in *T. denticola*.

Motility and chemotaxis are also involved in bacterial environmental responses. Oral treponemes have complete chemotaxis systems, with up to 2% of the total genome in *T. denticola* being devoted to chemotaxis and flagella [16]. The

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chemoreceptor component (methyl-accepting chemotactic protein (CP)) of the system monitors the environment, leading to signal transduction resulting in flagellar movement. *T. denticola* has over 20 genes encoding CPs [16], reflecting the complex niche that oral spirochaetes occupy. Serum, albumin and glucose, substances whose levels are increased in the diseased periodontal pocket, are chemotactic for oral treponemes *in vitro* [40,41]. The chemosensor DmcB was also identified as part of an environmental 'core stress response', confirming the importance of chemotaxis in response to environmental changes [30].

Mobile Elements and Genetic Exchange in Oral Spirochaetes

Bacteria in the periodontal pocket form biofilms, an ideal environment for genetic exchange [42]. Some treponemes harbour plasmids, such as pTS1, which has been found in both *T. denticola* and *T. socranskii* isolated from the same patient, suggesting the possibility of DNA transfer among species in the periodontal pocket [43]. Intergenus genetic transfer has also been demonstrated between *T. denticola* and the early biofilm colonizer Streptococcus gordonii [44]. Shuttle plasmid transformation from *T. denticola* to *S. gordonii* occurred in broth culture and artificial biofilms.

More recently, a microarray study of gene expression changes in planktonic and biofilm *T. denticola* cultures found a family of transposases within the genome [45]. Thirty-five genes with similarity to the IPR010106 domain found in known transposases are present in the *T. denticola* 35405 genome, 70% of which are upregulated in biofilm cultures. These elements may be involved in internal chromosomal rearrangement or horizontal gene transfer. A functional temperate bacteriophage, φtd1, was also isolated, and prophage gene expression was increased in biofilms. φtd1 may also play a role in horizontal gene transfer, as many of its genes can be traced to pathogens such as *Yersinia pestis* and other bacteriophages [45]. Additional regions of unusual DNA composition representing phage remnants, along with a 'clustered regularly interspaced short palindromic repeat' (CRISPR) locus together with adjacent CRISPR-associated genes, thought to be a mobile element, occur in *T. denticola* [16]. The presence of multiple elements involved in lateral DNA transfer suggests that genetic exchange is important for *T. denticola* survival in the periodontal biofilm.

Intragenomic recombination within the *T. denticola* genome also needs to be considered, as the genome contains redundancies and duplications [16], together with multiple variable segment regions, including CRISPR-associated regions, which have been suggested to be 'hot spots' for homologous recombination [46]. It is well established that other spirochaetes, such as *Borrelia burgdorferi*, are able to adapt to their multi-host environment and evade host immune responses by intragenomic recombination of silent cassettes, allowing for antigenic variation and switching of virulence genes [47].

Adhesion and Proteolytic Mechanisms of Oral Treponemes

Colonization of the oral cavity and formation of the multispecies dental plaque biofilm requires adherence to other microorganisms in addition to host proteins [42,48]. *T. denticola* co-aggregates with oral bacteria, including *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Tannerella forsythia*, through interactions involving spirochaete surface proteins [49–51]. *P. gingivalis* cell surface components such as fimbriae and haemagglutinins, together with the proteases, gingipains, also contribute to bacterial adhesion [48,52]. Recently, involvement of *P. gingivalis* ligands in co-aggregation with *T. denticola* has been further investigated, and the haemagglutinin domains (Hgp44) of gingipains and haemagglutinin A are key ligands for co-aggregation between these organisms [53]. A number of Gram-negative bacteria, including *P. gingivalis* and *T. denticola*, produce outer membrane vesicles (OMVs) [54,55], potent virulence factor 'packages', which can also aid in bacterial co-aggregation [54,56]. *P. gingivalis* is able to preferentially package gingipains in OMVs while excluding other abundant membrane proteins [57], implicating OMVs in *T. denticola* co-aggregation.

Adherence to human cells and extracellular matrix (ECM) is the first step in tissue penetration and resultant pathogenesis. Intact treponemes are able to bind to epithelial cells (*T. denticola*, some *T. socranskii* subspecies, *T. pectinovorum* and *T. lecithinolyticum*) [58–60], fibroblasts (*T. denticola* and *T. lecithinolyticum*) [61,62], endothelial cells (*T. denticola*, *T. socranskii* and *T. vincentii*) [63] and the ECM proteins laminin, fibronectin and collagens [59,63–65]. *T. denticola* possesses specific adhesins, including the major outer sheath protein (Msp), the oligopeptide transporter unit OppA and the chymotrypsin-like protease dentilisin [66–68].

OppA binds soluble fibronectin and plasminogen but not immobilized forms and, unlike other spirochaete surface proteins, it is not cytotoxic to epithelial or fibroblast cells. It has been proposed that, rather than undergoing direct host cell-binding interactions, OppA binds soluble matrix proteins to

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the bacterial surface as a means to evade the host immune response. OppA is also involved in peptide uptake and thus, indirectly, bacterial survival. It is present in *T. denticola* and *T. vincentii* but not in *T. socranskii* and *T. pectinovorum*, reflecting the differing metabolic requirements between *Treponema* species. A 52-kDa fibronectin-binding protein has also been identified in *T. lecithinolyticum*; it binds soluble and immobilized fibronectin [46], suggesting involvement in adhesion in both serum and tissue.

Recently, a comparative sequence analysis strategy used the *Treponema pallidum* fibronectin-binding protein Tp0155 to identify seven additional fibronectin-binding orthologues in *T. denticola* [69]. Of these, five were further analysed, and found to bind both matrix and plasma fibronectin. All members of this family contain M23 peptidase domains, and four members also contain LysM domains. M23 peptidases are able to degrade peptidoglycan, whereas LysM domains bind to carbohydrate polymers such as peptidoglycan. These features suggest that, in addition to fibronectin binding, these proteins may be involved in bacterial cell adhesion and peptidoglycan-modifying functions. Importantly, these multifunctional proteins may play a role in the lysis of other bacteria in the periodontal pocket, allowing for nutrient acquisition and furthering the survival of *Treponema* [69].

Msp is part of an outer sheath complex in *T. denticola*; it has both adhesin and porin properties [67,70,71]. Msp is also found in *T. vincentii*, but not in other oral spirochaetes [72,73]. 'Msp-like' homologues have been described in *T. maltophilum* and *T. socranskii* (MspA) [73], *T. lecithinolyticum* (MspTL) [74] and *T. pectinovorum* (MompA) [60]. Although 'Msp-like' loci and proteins are heterogeneous among phylotypes and *T. denticola*, they share many structural characteristics. They are all heat-modifiable, detergent-resistant, and protease-resistant. Like Msp [67,75], MspA, MspTL and MompA localize to the outer sheath [60,74,76]. Whereas the impact of Msp on host cells has been studied extensively, the role of 'Msp-like' proteins is unclear.

Proteases are crucial for tissue invasion as well as evasion of host defences. Dentilisin (PrtP) is distributed among *T. denticola*, *T. vincentii*, *T. putidum*, *T. socranskii* and *T. lecithinolyticum*, but is absent in *T. maltophilum* and *T. parvum* stains [77,78]. Sequence analysis of *prtP* and upstream *prcA* indicated two paralogous families on the basis of substrate specificity and bacterial phylogeny [77]. Dentilisin degrades diverse substrates, including ECM proteins, immunoglobulins, α_1-antitrypsin, complement C3, bioactive peptides and cytokines [79–83], suggesting involvement in bacterial adhesion, tissue penetration and immune evasion.

As *T. denticola*, *T. vincentii* and *T. putidum* are asaccharolytic [11,84], peptidases are vital for their nutrient acquisition. Various peptidases and peptidase activities have been characterized *in vitro* [85–90], but their pathogenic roles are not yet known. Recently, Ishihara *et al.* [91] have reported a cysteine protease, dentipain, in the *T. denticola* genome, a homologue of *Streptococcus pyogenes* IdeS. Its characterization revealed an enzyme with narrow specific oligopeptidase activity, cleaving only the β-chain of insulin. Notably, a dentipain-deficient mutant showed reduced skin abscess formation in a murine model.

Oral Spirochaetes Impact on Host Cells

Periodontal tissue cells

T. denticola cells and individual virulence factors are cytotoxic to epithelial cells [75,92]; they perturb cytoskeletal dynamics [93–98] and cell–cell junctions [99,100]. *T. denticola* can penetrate epithelium [96,101], whereas *T. medium* also invades epithelial cells [102]. Penetration of tissues by oral treponemes involves direct motility and proteolysis. Chemotaxis and flagellar mutants have impaired penetration [101], whereas a dentilisin mutant was unable to disrupt cell junctions or penetrate tissue layers [99,100].

Like all spirochaetes, oral treponemes have a unique structure and motility that affect their pathogenicity. Between their cytoplasmic membrane and outer sheath is a periplasmic space containing peptidoglycan and periplasmic flagella that extend from basal bodies at one pole towards the other pole. Beneath the cytoplasmic membrane, parallel to the flagella, are cytoplasmic filaments [103–106] (Fig. 1), providing treponemes with their distinctive 'wavelike' shape and movement. In addition to structural functions, cytoplasmic filaments are also involved in *T. denticola* biofilm formation as well as colonization of preformed *P. gingivalis* biofilms [107]. With the use of cryo-electron tomography, the natural cellular architecture of *T. denticola* has recently been refined by Izard *et al.* [108]. They identified novel periplasmic 'linkage' structures of dividing cells and cell-tip 'cone' structures (Fig. 1). Similar cone structures are present in other spirochaetes [108–110], but their structures vary, reflecting differing ecologies and pathogenic potentials.

Immune cells

Oral spirochaetes induce innate and adaptive immune responses. Systemic antibody responses towards treponemes, Msp and dentilisin are observed in sera of patients with periodontitis [31,111]. Periodontal diseases also involve innate immune responses of neutrophils and macrophages, cells that are affected by spirochaetes. Msp, MspA and

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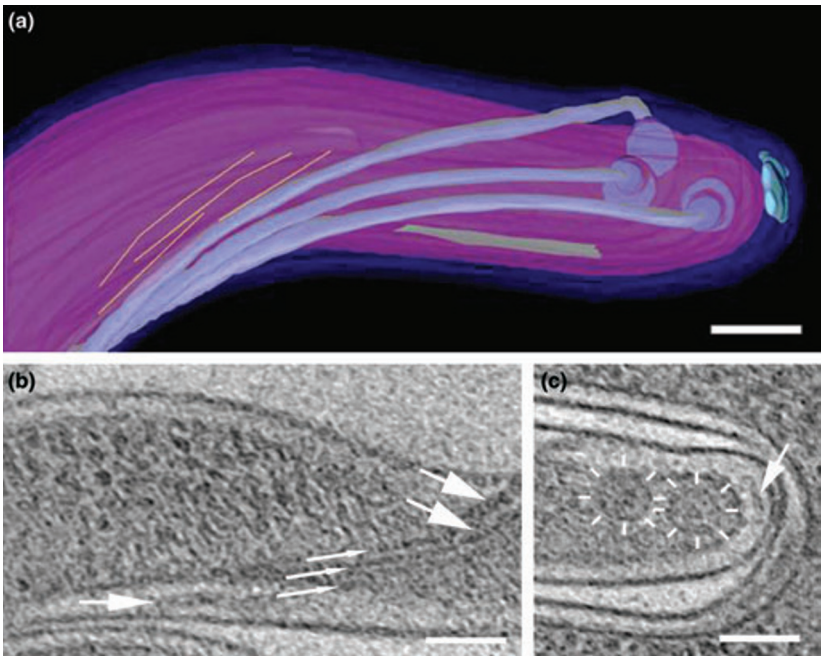

FIG. 1. Structure of *Treponema denticola*. (a) Surface-rendered model of *T. denticola*. Periplasmic flagella emerge from basal bodies (blue) and extend towards the cell centre. Cytoplasmic filaments (yellow) run parallel to the flagella, initiating from an attachment plate structure (grey). A periplasmic patella-shaped cone structure (light blue) is present at the cell tip. The outer membrane is dark blue and the cytoplasmic cylinder is purple. Scale bar: 100 nm. (b) Cytoplasmic filaments (thin arrows) and flagellar filaments (thick arrow) are depicted in a tomographic z-slice. The slice is 1.8 nm thick. Scale bar: 100 nm. (c) Lower rings of the flagellar basal bodies (radial lines) are depicted, along with the patella-shaped cone structure (arrow) at the cell tip in a tomographic z-slice. The slice is 1.8 nm thick. Scale bar: 100 nm. Images reprinted from *Journal of Structural Biology*, 163, Izard, J. *et al.*, Native cellular architecture of *Treponema denticola* revealed by cryo-electron tomography, p. 10–17. Copyright (2008), with permission from Elsevier.

MspTL induce the production of interleukin (IL)-6, IL-8, tumour necrosis factor-α, interferon-β and IL-1β by monocytes or macrophage-like cell lines [112–115]. Also, peptidoglycan, glycolipids and lipoproteins of *T. denticola* or *T. maltophilum* [115–118], along with the surface protein Td92, which is conserved among many oral phylotypes [119], induce monocytic cytokine production. Oral treponemes and membrane components also induce IL-6, IL-8, MCP-1, interferon-β and tumour necrosis factor-α production by epithelial cells and fibroblasts [113,120–124].

Toll-like receptors (TLRs) are key pathogen recognition molecules that lead to the transcription of inflammatory mediators. *T. denticola*, *T. vincentii* and *T. medium* and their outer membrane extracts activate TLR2 signalling in gingival epithelial cells [120]. Macrophage activation also occurs through TLR2 for Msp and through TLR4 for lipooligosaccharide [115]. Recognition of trepomemal glycolipids occurs through TLR2 [116], whereas MspTL stimulation of host cells appears to be TLR-independent [113]. Although treponemes activate TLR pathways, there is evidence that they may also mediate immune tolerance. Glycolipids from *T. medium* or *T. socranskii* and phospholipids of *T. denticola* or *T. medium* can inhibit host cell activation by other periodontal bacteria or *Escherichia coli* lipopolysaccharide [120,121,125,126], owing to inhibition of CD14 and lipopolysaccharide-binding protein interactions with TLR [121,125]. Moreover, Msp and lipooligosaccharide can induce macrophage tolerance through TLR4 [115]. The ability of oral treponemes to dampen immunity to other bacteria is intriguing, considering the polymicrobial nature of periodontitis.

T. denticola can impair some neutrophil functions *in vitro*. It was reported to inhibit superoxide production in human neutrophils [127]. Recent studies have focused on *T. denticola* Msp inhibition of neutrophil polarization and chemotaxis in chemoattractant gradients, through selective inhibition of the small GTPase Rac1 [128]. Msp also perturbs actin assembly [93,129], calcium transients and phagocytosis [129].

Endothelium

The impact of oral treponemes on the endothelium is less well understood. Leukocyte infiltration occurs during chronic

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periodontitis [130], as does systemic dissemination of oral spirochaetes [131]. Oral treponemes can attach to endothelial cells [63]. *T. denticola* and outer membrane preparations perturb porcine endothelial cell homeostasis by inducing apoptosis and expression of heat shock proteins [132]. Also, MspTL is able to increase adhesion of monocytes to endothelial cells and transendothelial migration [122]. Surface components of treponemes probably contribute to leukocyte infiltration into periodontal tissues, and subsequent tissue injury.

In vivo models

Early models used to study oral treponeme pathogenicity *in vivo* involved murine subcutaneous abscess formation as a measure of tissue damage [133]. However, more recently, murine and rat models of oral infection have been developed that accurately reflect the site of colonization, alveolar bone loss and immune response characteristics of periodontal disease [134,135]. These models have been used to study alveolar bone loss in both monomicrobial and polymicrobial infections [134], as well as to characterize the systemic immune response and identify potential bacterial antigens responsible, such as Msp and dentilisin [135].

Host transcriptional profiles during *T. denticola* infection in a murine calvarial model of inflammation and bone resorption have also recently been examined [136]. Numerous biological pathways were affected, including inflammatory mediators, cell adhesion, ECM interactions and cell cycle components. This study corroborated the results of many *in vitro* studies, as well as identifying additional host pathways perturbed by *T. denticola*.

Concluding Remarks

Oral spirochaetes occupy a unique niche in terms of environment and their polymicrobial nature. Treponemes possess a wide range of virulence factors that promote survival and pathogenicity in the gingival pocket. Recent examples of mobile DNA elements, genetic exchange and bacteriophages highlight the complexity of interactions between organisms in the oral cavity. Recent research has also focused on how oral treponemes sense and respond to the dynamic environments. They have multiple TCSs and chemotaxis-sensing receptors, and may respond by locomotion and virulence expresssion. They also express multiple uptake and regulatory systems for nutrient acquisition. Oral spirochaetes affect multiple host cell types. Notably, they can activate immune responses, leading to tissue injury, but impair some crucial innate responses, including neutrophil function and TLR activation, preventing their own eradication. Finally, oral treponemes have many conserved as well as some unique virulence properties. Progress in molecular tools, cultivation and genome analysis will undoubtedly encourage further advances in understanding their role in periodontal diseases.

Transparency Declaration

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