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Research Article

C. Albicans Biofilm Formation is Restricted by Periodontal Ligament Cells and Induces Differential Cytokines Response Compared to Planktonic *C. Albicans*

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Abstract

Candida albicans is the most numerous commensal and potentially pathological yeast in the human oral cavity. Here we investigate the ability of *C. albicans* to form a biofilm on human root tip surfaces (RTs) in the presence of periodontal ligament (PDL) cells, and we compare the expression of cytokines in PDL cells induced by biofilm on RTs compared to that induced by planktonic *C. albicans*. PDL cells were added at the adhesion phase of *C. albicans*, or after *C. albicans* cells were allowed to attach to RTs for 90 minutes. The whole mixed culture was allowed to form a biofilm. Development of a biofilm on RTs was analyzed after 24 h using the XTT reduction assay and by scanning electron microscopy. To analyze cytokine expression by real-time PCR at two and three hours, biofilms formed on RTs or planktonic *C. albicans* were co-cultured with PDL cells. Our results show that PDL cells limit biofilm formation, especially when co-cultured before the attachment phase. Biofilms induced a more persistent expression of IL-1 β and RANKL in PDL cells than did planktonic *C. albicans*. IL-10 expression in PDL cells was down regulated in response to biofilms or planktonic *C. albicans* as compared with controls.

Keywords: *Candida albicans* biofilm; Inflammatory cytokines; Periodontal ligament cells; Planktonic *C. albican*

Introduction

Microorganisms are associated with all diseases of the dental pulp that lead to periradicular lesions [1,2]. Many studies have documented the presence of fungi in endodontic infections, and its role in the aetiology of periradicular lesions [3-8].

Several fungi are considered normal microbial inhabitants of the oral cavity, but may result to disease when there are local or systemic factors predisposing the individual to infection. *Candida albicans* is considered the most frequent commensal and sometimes pathogenic yeast in the oral cavity [9]. It has been reported that 21% of infected root canals contain *C. albicans* [6]. A pure culture of *C. albicans* has been found to be the causative factor of an acute apical abscess [10]. *C. albicans*, *Enterococcus faecalis* and *Actinomyces* are the most prevalent microorganisms associated with persistent secondary endodontic infections that do not respond to current root canal therapy [11-14]. These reports support that *C. albicans* are involved in the aetiology of persistent periradicular lesions. There are multiple mechanisms involved in the pathogenicity of *C. albicans*. Adaptation and adhesion to a variety of surfaces, including dentine and root surfaces, is believed to be a crucial virulence factor [8-12].

Many oral microbes form and live within a biofilm matrix composed of exopolysacchardies, proteins, and nucleic acids that protect them from the environment and immune system. Biofilm formation, leading to immune-evasion and immune-modulation of the host defense, is considered another key virulence factor of *C. albicans*. Formation of the biofilm can provide to the *C. albicans* community protection against antimicrobial agents compared to those in a nomadic state (e.g. planktonic cells). *C. albicans* in biofilm can be 100-fold more resistant to antifungal fluconazole and 20- to 30-fold more resistant to antifungal amphotericin B than planktonic cells [15]. Interestingly, biofilm formation is associated with the longstanding pathological process of periapical lesions [16]. Heavy colonization of yeast cells was reported in teeth with persistent periapical lesion [5,12]. The availability of a well-characterized, reproducible biofilm model is essential in understanding the nature of *C. albicans* biofilm pathogenicity on the root surface in order to develop a more effective treatment strategy.

Periodontal ligament (PDL) cells play a crucial role in the early infection as well as resolution stage of infection at root surfaces [17]. They respond to infection by secreting potent pro-inflammatory cytokines, such as Interleukin (IL) β -1 (IL-1β), IL-6 and tumor necrosis factor-α (TNF- α). These cytokines promote inflammation and induce Receptor activator of nuclear factor kappa-B ligand (RANKL) secretion, that leads to osteoclast differentiation and bone resorption. IL-17 is another proinflammatory cytokine secreted by a subset of CD4+ T helper cells, named Th17 cells. It has been shown that Th17 cells are involved in several inflammatory diseases including chronic lesions of human periodontal disease [18]. Th17 cells are present in high numbers in induced rat periapical lesions [19]. Interestingly, the IL-17 receptor (IL-17R) is expressed by human PDL cells [20], suggesting that PDL cells respond to Th17 cells of adaptive immunity to promote inflammation. It should be noted that Th17 cells are known to play a protective role against *C. albicans* infection

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in the oral cavity [9]. Therefore PDL cells may play an integral role in the resistance to *C. albicans* infection at the expense of exacerbating inflammation and promoting periapical lesions. Thus, studying the interaction between *C. albicans* infection and periodontal cells should advance current understanding of the mechanism involved in the aetiology of persistent periradicular lesions related to fungal infections.

PDL cells secrete cytokines such as IL-10 and transforming growth factor-β (TGF-β), which are anti-inflammatory and necessary for tissue repair during the healing process [21]. Therefore, studying the immune response of PDL cells against *C. albicans* may help to determine the fate of the inflammatory response as it relates to tissue destruction versus tissue repair during and following infection.

Material and Methods

Cell Culture, *C. albicans*, and growth conditions

Primary human PDL fibroblasts isolated from human periodontal tissues were obtained from ScienCell (Carlsbad, CA) and grown in complete culture medium; Dulbecco's modified Eagle's medium medium (Invitrogen, CA) supplemented with antibiotics and 10% fetal bovine serum (FBS), at 37°C in a humidified 5% CO_2 atmosphere. Cells between the 4th and 6th passages were used in the present study.

The *C. albicans* wild type SC5314 (courtesy of Dr. Ken Nickerson, University of Nebraska, Lincoln) were grown in yeast nitrogen base (YNB) medium (Difco Laboratories, Detroit, Mich.) from fresh Sabouraud dextrose agar plates (Difco) and incubated for 24 h at 37°C in a shaker at 60 rpm. Cells were harvested and washed twice with phosphate buffered saline (PBS). Cells were then re-suspended in 10 mL of PBS, counted following serial dilution, standardized, and used within 24 h.

Biofilm formation on the root tip surface

The study protocol was reviewed by the Institutional Review Board (IRB#022-10-EP). Freshly extracted human single intact root tip surfaces (RTs), with an unknown history, from maxillary and mandibular anterior teeth were sectioned approximately 4-mm in length, placed into 6% sodium hypochlorite solution for 24 h to remove any stains, and autoclaved. They were then placed onto 12-well tissue culture plates and incubated in FBS for 24 h at 37°C. Following incubation in the FBS; pretreatment phase [22], they were transferred to new plates and washed with PBS to remove residual FBS, and were immersed in a *C. albicans* suspension containing 10^7 cells/mL. Finally, they were incubated in 5% CO_2 at 37°C to allow biofilm formation over the external root surfaces for 24 h. As controls, RTs were processed in an identical fashion, but without the addition of *C. albicans*. These samples were used later to analyze cytokine expression of PDL cells in response to planktonic *C. albicans* cells or biofilm form.

Quantitative measurement of biofilms, scanning electron and light microscopy

Biofilm formation by *C. albicans* on the RTs in the presence of PDL cells was investigated using a colorimetric assay and scanning electron microscopy (SEM). PDL cells (2×10^6) were added either at the adhesion phase of *C. albicans*, or after *C. albicans* were allowed to attach to RTs for ninety minutes, as described previously [23]. The whole mixed culture, in complete culture medium; Dulbecco's modified Eagle's medium (Invitrogen, CA) supplemented with antibiotics and 10% fetal bovine serum (FBS), was then allowed to form a biofilm.

Following maturation for 24 h, biofilms were quantified by tetrazolium-salt-based (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino) carbonyl]- 2H-tetrazolium hydroxide (XTT) as described previously [24].

Root tip surfaces with biofilms were transferred to 12-well tissue culture plates containing 4 mL of PBS per well. Empty wells and RTs co-cultured with PDL cells were used as negative controls. *C. albicans* cultured alone served as positive control. Fifty uL XTT (1 mg/mL in PBS) and 4 uL menadione solution (1 mM in acetone) were added to each well. Plates were incubated at 37°C for 5 h. The contents of each well, excluding RTs, were transferred into 96-well culture plates. A water soluble brown formazan product was determined spectrophotometrically at 492 nm.

In a parallel experiment, RTs were prepared for SEM. They were fixed with 10% formalin, followed by fixation with osmium tetraoxide. This was followed by a series of ethanol dehydration steps, and the prepared samples were sputter coated with Au-Pd (60/40 ratio) and viewed with a model JEOL 6100 microscope.

To visualize PDL cells, cells were fixed in 10% formalin and subjected to light microscopy analysis.

Cytokine expression of PDL cells in response to planktonic *C. albicans* cells or Biofilm form

After the PDL cells reached confluence in culture medium, they were collected, washed, and counted with a haemocytometer. A total of 2×10^6 cells were plated in culture medium onto six-well plates and grown overnight in 5% CO_2 at 37°C to allow adherence to the surface. On the next day, the non-adherent cells were removed from the plates by aspiration and fresh complete culture medium was added. Culture plates containing PDL cells were then divided into the following groups:

Group (1) PDL cells alone were maintained in complete culture medium (Control).

Group (2) PDL cells were co-cultured with root tips with no biofilm (Control).

Group (3) PDL cells were challenged with planktonic *C. albicans* at ratio (1:1) based on seeding cell densities.

Group (4) PDL cells were co-cultured with root tips that were exposed to *C. albicans* for 24 h to form biofilms as described above. After 2 and 3 h, RNA was extracted based on preliminary data (not shown).

RNA isolation, cDNA, and quantitative RT-PCR

To quantify the expression of cytokines by PDL cells, RNA was extracted using the PureLink RNA Mini Kit (Invitrogen, CA) according to the manufacturer's instructions. cDNAs were prepared from 1 μg of RNA using standard protocol [25]. Quantitative real-time PCR (qRT-PCR) was performed with the Power Sybr Green PCR Master (Invitrogen, CA). Quantitative PCR reactions were run on an ABI Prism 7000 thermal cycler in which 1 μL of cDNA with primers was incubated at 95°C for 10 minutes, followed by 40 cycles

of 95°C for 15 seconds, and 60°C for 30 seconds. Cycle thresholds (Ct) were normalized to Ct for GAPDH for each cDNA and were expressed by fold increase using the formula: $2^{-\Delta\Delta Ct}$. The sense/antisense primers were designed using the Oligo Perfect Designer software (Applied Biosystems, Ca, USA) based on published human upstream and downstream sequences of each gene's cDNA derived from mRNA. The sense/antisense primers used of IL-1β, IL-6, IL-10, TNF-α, RANKL, IL-23 p19, IL-1L-7R, TGF-β1 and GAPDH were listed in table 1

Statistical Analysis

Each experiment was independently performed three times in duplicate on separate days. One-way analysis of variance was performed to compare means of multiple groups, and the one-tailed Student's t-test was used for analysis of two groups. Results with a P-value less than 0.05 were considered statistically significant.

Results

PDL cells limit biofilm formation

It was hypothesized that PDL cells influence the immune response and the ability of *C. albicans* to form a biofilm. Therefore, PDL cells were co-cultured immediately before the attachment phase or ninety minutes after *C. albicans* were allowed to attach to the RTs. By utilizing the metabolic activity of cells (XTT reduction assay), it was shown that PDL cells limited the biofilm formation significantly by 40% if co-cultured immediately, and by 25% if *C. albicans* were first allowed to attach to RTs (Figure 1). In both instances, the difference in means was highly significant ($p<0.05$) compared with biofilm formed by *C. albicans* alone (Figure 1). The data represents pool of three experiments performed in duplicate.

Using SEM to visualize biofilm formation on RTs revealed that *C. albicans* formed a hypha-rich biofilm over the entire external RTs surface in the absence of PDL cells (Figure 2a). The effect of PDL cells was obvious when they were co-cultured immediately (Figure 2b). However, when *C. albicans* were allowed to attach before co-culturing with PDL cells, a hypha biofilm formed over the external RTs surface when examined with SEM (Figure 2c). Nevertheless, there was less *C. albicans* hypha-rich biofilm in the presence of PDL cells.

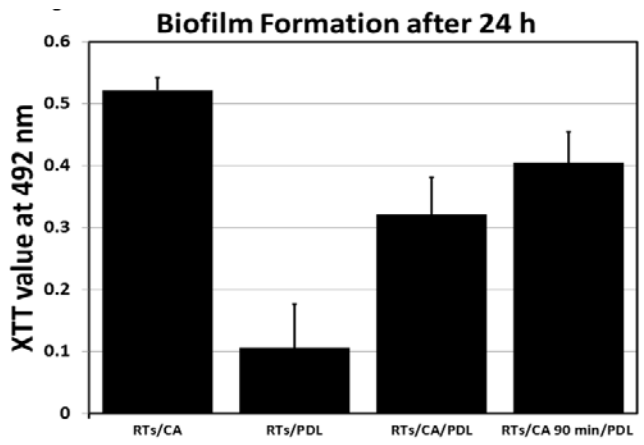

Figure 1: Metabolic activity of *C. albicans* biofilms formed in different experimental groups is presented as means ± standard error (SE) of optical density at 492 nm.

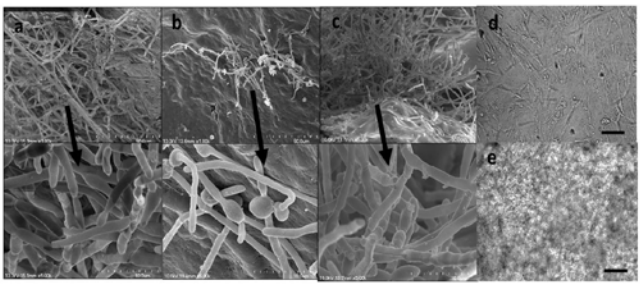

Figure 2: SEM analyses at accelerating voltage of 10 KV of the effect of PDL cells on *C. albicans* grown as (a) biofilms formed by *C. albicans* alone on RTs, (b) with PDL cells co-cultured immediately, or (c) with PDL cells co-cultured after *C. albicans* were allowed to attach for 90 minutes. *C. albicans* formed hypha-rich biofilms in the absence of PDL cells. Scale bar and magnification are shown. Light microcopy images of PDL cells co-cultured with RTs (d and e). PDL cells had normal morphology in absence of *C. albicans* (d), and a layer of biofilm formation covering wells with no evidence of PDL cells when *C. albicans* were added. Scale bar = 100 μm.

Light microscopy was used to visualize PDL cells in wells containing RTs that were co-cultured with *C. albicans*. It is noted that wells became covered with a layer of biofilm but PDL cells could not be seen (Figure 2e). PDL cells had normal morphology in wells that contained RTs with no *C. albicans* (Figure 2d).

PDL cells have a differential inflammatory response to planktonic C. albicans as compared with biofilm form on RTs

It was hypothesized that PDL cells express different or different levels of inflammatory cytokines in response to *C. albicans* as planktonic cells when compared to biofilm. As shown in Table 2, compared to the level of mRNA expressed from the same number of PDL cells grown alone or with root tips, the level of proinflammatory factors IL-1β, RANKL, IL-6, TNF-α, IL-23p19 and IL-17R was up-regulated from the PDL cells co-cultured with planktonic *C. albicans* or RTs with biofilm at 2 and 3 h of stimulation. However, IL-1β and RANKL, factors known to induce bone resorption, were persistently up-regulated at 3 h in response to RTs with biofilms, in contrast to of planktonic *C. albicans*. The difference in means was significant ($p<0.05$). Both IL-6 and TNF-α expression were significantly greater ($p<0.05$) in response to planktonic *C. albicans* at 3 h compared with biofilms.

As shown in table 2, IL-17R and IL-23p19 mRNA were expressed in response to planktonic *C. albicans* and RTs with biofilms. Interestingly, the induction of IL-23 p19 subunit was significantly greater ($p<0.05$) at 2 h in response to planktonic *C. albicans* as compared to RTs with biofilms (Table 2). However, the expression was decreased to comparable levels in response to planktonic *C. albicans* and RTs with biofilms at 3 h (Table 2). In contrast, IL-17R mRNA was expressed at significantly higher levels ($p<0.05$) in response to RTs with biofilm at 2 h compared with planktonic *C. albicans*. The expression of IL-17R mRNA was at the same level in response to planktonic *C. albicans* and RTs with biofilms at 3 h.

Finally, the expression of the key anti-inflammatory cytokines during the healing process was analyzed. IL-10 mRNA expression was constitutively expressed by PDL cells, and was down-regulated significantly ($p<0.05$) by planktonic *C. albicans* and RTs with biofilms

Table 1: The sense/antisense primers used for real time PCR.

TARGET	SENSE PRIMER	ANTISENSE PRIMERS
IL-1β	5′-ACGCTCCGGGACTCACAGCA	5′-TGAGGCCCAAGGCCACAGGT
IL-6	5′-AGGAGACTTGCCTGGTGAAA	5′-CAGGGGTGGTTATTGCATCT
IL-10	5′-TGGTGAAACCCCGTCTCTAC	5′- CTGGAGTACAGGGGCATGAT
TNF-α	5′-TCCTTCAGACACCCTCAACC	5′-AGGCCCCAGTTTGAATTCTT
RANKL	5′-GTCTGCAGCGTCGCCCTGTT	5′-ACCATGAGCCATCCACCATCGC
IL-23P19	5′-GTTCCCCATATCCAGTGTGG	5′-TTTTGAAGCGGAGAAGGAGA
IL-17R	5′- CACTAGCCTTTTGGGCTCAG	5′- TACGCAGGAAGAGTGCATTG
TGF-β1	5′- GGGACTATCCACCTGCAAGA	5′- CCTCCTTGGCGTAGTAGTCG
GAPDH	5′-CAGCCTCCCGCTTCGCTCTC	5′- CCAGGCGCCCAATACGACCA

at 2 and 3 h. However, TGF-β1 was highly expressed ($p<0.05$) only in response to planktonic *C. albicans* compared to RTs with biofilm infection at 2 and 3 h (Table 2).

Discussion

Current *Candida* biofilm models use sections of synthetic clinically relevant materials. An *ex vivo* model is necessary to study *C. albicans* biofilm infection of root surfaces, which are involved in the aetiology of persistent periradicular lesions [11,12]. Furthermore, *C. albicans* infections have to overcome the immune response mediated by supporting tissue and cells of roots. Therefore, in the present study an *in vitro* biofilm model was developed to investigate the ability of *C. albicans* to form a biofilm on human root surfaces in the presence of periodontal ligament cells, and to compare cytokine expression of PDL cells in response to biofilm compared with planktonic *C. albicans*. The current study demonstrated that PDL cells limit the formation of biofilms by *C. albicans* on the root surfaces. To our knowledge, this is the first report to study the interaction between PDL cells, and *C. albicans* and its ability to form a biofilm on root surfaces. Classic studies have indicated that teeth with periapical lesions have lower success rates compared to ones without [26-28]. Biofilms are present and associated with the longstanding pathological process of periapical lesions [16]. Given the fact that all current non-surgical endodontic therapy addresses the intra-canal infection, it is ultimately the host's responsibility to clear extra-canal infections and biofilms. Therefore, it is important to establish an *ex vivo* model that simulates a clinical scenario [10]. In this study, *C. albicans* formed biofilms on root surfaces and interacted with host cells.

The present study was motivated by Chandra et al. (2007), who demonstrated that co-cultured *C. albicans* with blood mononuclear cells (BMCs) enhanced biofilm formation [23]. In their study, *C. albicans* were allowed to adhere to synthetic discs prior to co-culturing with BMCs. There were few monocytes visible on the surface of the biofilms and they failed to phagocytize *Candida* cells. In contrast, BMCs incubated with planktonic *C. albicans* were phagocytized. It is generally accepted that the phagocytosis-induced apoptosis of BMCs and subsequent clearance by macrophages is a prerequisite for the resolution of infection-associated inflammation, while the failure to undergo apoptosis creates a pathological situation [29,30]. Studies have reported the occurrence of *C. albicans* in primary and persistent endodontic infection [12]. One aspect of *C. albicans* pathogenicity is related to its ability to form biofilms [31]. Biofilm formation has been described as a production of filamentous scaffolding and initial deposition of extracellular matrix material [32]. *C. albicans* biofilm exhibits low metabolic activity in the first 6 h, which then increases exponentially until maturity is reached by 24 h [24,33]. For *C. albicans* to form a biofilm, it must interact with and attach to the supporting structure and cells of root surfaces. Further analysis is needed to explore the dynamic interactions between PDL cells and fungal biofilms. Therefore, a system was developed to test this hypothesis. Utilizing quantitative assay (XTT), it was shown that PDL cells limited *C. albicans* biofilm formation. However, once *C. albicans* attached to the root surfaces during the initial phase of biofilm formation, the presence of PDL cells were less effective. The SEM analysis was conducted to visualize the morphology and architecture of biofilms. It was clear that the presence of PDL cells resulted in less mature biofilms over root surfaces. While the mechanism of how PDL cells limit biofilm formation before or after *C. albicans* attaches

Cytokines	PDL		PDL/CA		PDL/RTs		PDL/biofilm	
	2 hr	3 hr	2 hr	3 hr	2 hr	3 hr	2 hr	3 hr
	CT value	CT value	CT value	CT value	CT value	CT value	CT value	CT value
IL-1β	0.00001±0.0001	0±0.00001	1.02±0.3	1.8±0.6	0.00001±0.001	0±0.00001	0.96±0.2	3.7±1.6
RNAKL	0.0003±0.0003	0.0003±0.0001	2.3±1	1.6±0.3	0.0001±0.0001	0.0001±.00001	0.9±0.2	5.75±1.12
IL-6	0.03±0.009	0.02±0.01	2.1±1.5	3.64±1.4	0.02±0.009	0.03±0.008	1.6±0.57	1.1±0.29
TNF-α	0.001±0.0007	0.001±0.0008	1.7±0.6	1.59±0.4	0.002±0.0008	0.002±0.0006	0.78±0.2	0.487±0.07
IL-23p19	0.0003±0.0002	0.0005±0.0001	4.3±1.5	0.67±0.0001	0.0008±0.0004	0.001±0.001	1.12±0.17	0.43±0.15
IL-17R	0.004±0.001	0.004±0.001	1.3±0.3	1.64±0.6	0.0005±0.001	0.006±0.001	1.8±0.66	1.35±0.4
IL-10	14.6±4.9	9.084	0.7±0.19	1.52±0.2	11.6±4	19.1±6.9	0.8±0.2	1.7±0.08
TGF-β	0.001±0.0003	0.0030.001	5.7±2.3	2.83±0.3	0.005±0.001	0.65±0.001	2.12±0.9	0.65±0.08

Table 2: Normalized Ct values of IL-1β, RANKL, IL-6, TNF -α, IL-23p19, IL-17R, IL-10, and TGF-β1 expression in response to planktonic *C. albicans* or root tips with biofilm. Real - time PCR of mRNA in PDL cells. PDL cells (2×10^6) alone, with 2×10^6 cells of *C. albicans* or RTs with or without biofilms at 2 and 3 h. Data represents mean of relative levels of 6 samples and 3 independent experiments.

to the root surfaces is yet unknown, it is clear that competent PDL cells promote resistance to *C. albicans* biofilm formation at the root surfaces of teeth during the attachment phase.

XTT assay has been used as a viability assay of different organisms including mammalian cells, bacteria and fungi [24,34,35]. In the present study, PDL cells alone also metabolized XTT (Table 1). However, signals produced by *C. albicans* biofilm in the absence of PDL cells were significantly higher (4-folds) than that of biofilm formed by *C. albicans* co-cultured with PDL cells (Table 1). Our findings show that PDL cells clearly exert anti-biofilm activity.

Results here demonstrate that the cytokine profile of PDL cells following co-culturing with planktonic versus biofilm *C. albicans* is different. For RT-qPCR, it is necessary that an appropriate housekeeping gene for normalization should be equally expressed under different conditions for accurate and reliable analysis. In the present study, the gene for glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) was chosen because it is an essential enzyme in all cells. However, GAPDH is also found at the cell surface of *C. albicans* [36]. The primers used here for RT-qPCR of human GAPDH do not have any homology to the GAPDH cDNA sequence of *C. albicans*.

Cytokines produced by PDL cells co-cultured with biofilms showed a pattern of persistently higher expression of IL-1β and RANKL cytokines, which are associated with bone resorption. The data is in agreement with the fact that biofilms are present and associated with the longstanding pathological process of periapical lesions [16]. The data also indicates that the expression of IL-10 is significantly down-regulated by planktonic *C. albicans* and biofilms, suggesting that these infections target this cytokine. Down-regulation of IL-10 may be a mechanism by which *C. albicans* promote inflammation from human immune cells such as PDL cells. IL-10 is a potent anti-inflammatory regulatory cytokine which decreases the expression of proinflammatory T cell cytokines such as IFN- and IL-17. It is reasonable to conclude that the decline in IL-10 contributes to the rise in expression of the proinflammatory cytokines IL-6, TNF-α and IL-, and the increase in expression of the IL-17 receptor.

IL-17 is a proinflammatory cytokine produced primarily by the Th17 subset of $CD4^+$ T cells. While its expression depends in part upon proinflammatory cytokines IL-1β and IL-6, one of its roles is to induce expression of proinflammatory cytokines such as IL-6 and TNF-α through the IL-17 receptor. These cytokines are also involved in osteoclastogenesis by inducing RANKL expression in osteoblasts [37]. The presence of Th17 cells and significantly higher levels of IL-17 expression have been found in periodontal lesions, with especially high levels at the lower lesional site adjacent to the sites of bone destruction [18,37]. IL-23 is an essential factor required for the survival and expansion of the Th17 cells and the production of IL- 17. In the present study, PDL cells expressed both IL-23 p19 and IL-17R in response to biofilm and planktonic *C. albicans*. However, the response was relatively higher at early stages which may favor bone resorption due to destructive feature of Th17 axis. In contrast to its role in several inflammatory pathologies, Th17 cells are known to play a critical protective role against *C. albicans* infection in the oral cavity [9]. Mice rendered deficient in IL-17 are significantly more susceptible to oropharyngeal infection with *C. albicans* [38]. Therefore, therapeutic inhibition of IL-17 axis to prevent bone resorption induced by *C. albicans* infection may not be efficacious.

Conclusions

The present studies have shown that PDL cells may limit the formation of a biofilm by *C. albicans*. Furthermore, PDL cells expressed distinct and differential proinflammatory cytokines in response to a biofilm compared with planktonic *C. albicans*. The current *ex vivo* model presented will allow the conduction of further studies to understand the pathogenicity of *C. albicans* biofilms on root surfaces of teeth, as well as the host response.

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