ORIGINAL RESEARCH

Evaluation of the effect of Doxycycline on S100A8 and S100A9 gene expression in human gingival fibroblasts induced by *Porphyromonas gingivalis* lipopolysaccharide

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Abstract

Introduction: S100A8 and S100A9 are alarmins which are primarily and increasingly expressed from granulocytes during inflammation. Gingival fibroblasts are the principal cells in the gingival connective tissue. In addition to collagen homeostasis, evidence points out that they are active in the host immune response towards periodontopathic microbes.

Aim and Objectives: This in vitro study aimed to explore if Porphyromonas gingivalis lipopolysaccharide (Pg-LPS) could induce gene expression of S100A8 and S100A9 from human gingival fibroblasts (HGF's). This study also assessed the effect of doxycycline (doxycycline hyclate) on the mRNA expression of these proteins.

Materials and methods: Human gingival fibroblast (HGF) cultures were established from healthy gingival tissue samples. 5µg/ml of Pg-LPS was added to the cell cultures and mRNA levels of S100A8 and S100A9 were assessed by quantitative polymerase chain reaction (qPCR). Cell viability was assessed by MTT assay. Following this, 0.1, 0.5, and 1 µg/mL of doxycycline were added to the culture and qPCR was repeated.

Results: There was a significant expression of S100A8 and S100A9 from HGF's, when induced by Pg-LPS. Addition of 0.1, 0.5, and 1 µg/mL doxycycline reduced the gene expression of these proteins from HGF's. Maximum inhibition of mRNA expression was seen at 0.1 µg/mL of doxycycline.

Conclusion: HGF's are active in host immune response against P. gingivalis and express S100A8 and S100A9. The gene expression of S100A8 and S100A9 are decreased by the addition of doxycycline to the cell cultures.

Key words: Alarmins, Gene expression, Doxycycline.

Introduction

Gingival tissues encompass the teeth and are constantly challenged by physio-chemical, mechanical and microbial stressors. In an individual susceptible to periodontal disease, dysbiosis in the plaque biofilm subjacent to the tooth, initiate periodontal inflammation.[1] Periodontal diseases lead to degradation of the periodontal ligament and alveolar bone, tooth mobility and tooth loss. Inflammation in the periodontal tissues can also contribute to low grade systemic inflammation.[2]

The gingiva is composed of the gingival epithelium and the underlying connective tissue. Gingival fibroblasts are the key cellular element of the gingival connective tissue which is predominantly constituted by collagen fibers. Gingival fibroblasts maintain integrity of collagen fibers by synthesizing new collagen and degrading the old by collagenases and phagocytosis. In addition to its classical role in the homeostasis of the extracellular matrix, it is now recognized that gingival fibroblasts are 'sentinel cells' with versatile and diverse immune functions.[3] Gingival fibroblasts exposed to pathogenic bacteria express a vast array inflammatory mediators and antimicrobial petides.[4]

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They also express pathogen recognition receptors such as Toll-like receptors (TLR's), Protease-activated receptor 1 (PAR-1) and Nucleotide-binding oligomerization domain-containing protein receptors. (NLR's)[3] Evidence has pointed out that Toll like receptor (TLR) mediated expression of pro inflammatory cytokines occurs in gingival fibroblasts when exposed to microbial components.[5,6] Thus gingival fibroblasts are 'non-classical' constituents of the innate immune system.[6]

Porphyromonas gingivalis (P. gingivalis) has been recognized as a key stone pathogen capable of inducing dysbiosis in plaque biofilms subjacent to teeth and other intra-oral hard surfaces such as dental restorations, implants, and fixed or removable intra oral prosthesis. P. gingivalis even at lower colonization levels can induce dysbiosis and in participation with the commensal flora in the biofilm, can disrupt the host-microbe homeostasis leading to periodontal diseases.[7] In addition to its role in inflammatory tissue destruction, P. gingivalis also evades host defense mechanisms.[8]

Dysregulated host immune response is initiated by pathogen or damage associated molecular pattern (PAMP or DAMP) binding to pathogen recognition receptors (PRR's) of the immune cells. The role of PAMP's such as LPS, flagellin and bacterial DNA are very well recognized in periodontal pathogenesis and there is a growing understanding on the potential role of DAMP's in inflammasome activation.[9] Contrary to the conventional understanding that the immune

Table 1: Primer sequences of S100A8 and S100A9

Primer	Forward	Reverse
S100A8	CCGAGTGTCCTCAGTATATCAGGA	GCCCATCTTTATCACCAGAATGA
S100A9	TCAAAGAGCTGGTGCGAAA	CAGCTGCTTGTCTGCATTTG
GAPDH	TTGGCTACAGCAACAGGGTG	GGGGAGATTCAGTGTGGTGG

Table 2: Mean fold changes in S100A8 mRNA levels

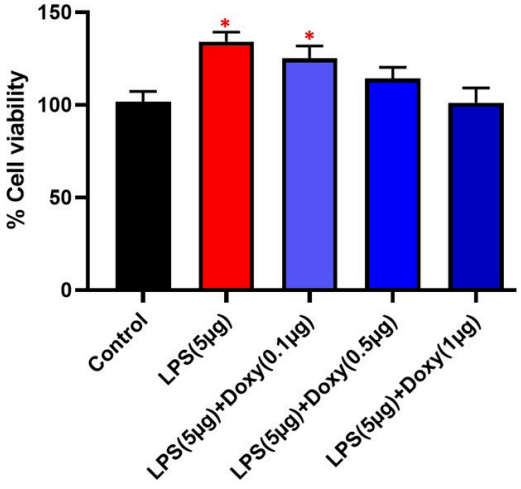
Groups	Group names	Mean Diff.	95.00% CI of diff	Significant?	Summary	Adjusted P Value
Control vs. LPS	A-B	-0.3633	-0.6438 to -0.08287	Yes	*	0.0112
Control vs. LPS+Doxy(0.1µg)	A-C	0.8400	0.5595 to 1.120	Yes	****	<0.0001
Control vs. LPS+Doxy(0.5µg)	A-D	0.5500	0.2695 to 0.8305	Yes	***	0.0005
Control vs. LPS+Doxy(1µg)	A-E	0.2800	-.0004617 to 0.5605	No	NS	0.0504
LPS vs. LPS+Doxy(0.1µg)	B-C	1.203	0.9229 to 1.484	Yes	****	<0.0001
LPS vs. LPS+Doxy(0.5µg)	B-D	0.9133	0.6329 to 1.194	Yes	****	<0.0001
LPS vs. LPS+Doxy(1µg)	B-E	0.6433	0.3629 to 0.9238	Yes	***	0.0001
LPS+Doxy(0.1µg) vs. LPS+Doxy(0.5µg)	C-D	-0.2900	-0.5705 to -0.009538	Yes	*	0.0420
LPS+Doxy(0.1µg) vs. LPS+Doxy(1µg)	C-E	-0.5600	-0.8405 to -0.2795	Yes	***	0.0005
LPS+Doxy(0.5µg) vs. LPS+Doxy(1µg)	D-E	-0.2700	-0.5505 to 0.01046	No	NS	0.0605

Table 3: Mean fold changes in S100A9 mRNA levels

Groups	Group names	Mean Diff.	95.00% CI of diff	Significant	Summary	Adjusted P value
Control vs. LPS	A-B	-0.8433	-1.162 to -0.5246	Yes	****	<0.0001
Control vs. LPS+Doxy(0.1µg)	A-C	0.8467	0.5279 to 1.165	Yes	****	<0.0001
Control vs. LPS+Doxy(0.5µg)	A-D	0.4100	0.09122 to 0.7288	Yes	*	0.0117
Control vs. LPS+Doxy(1µg)	A-E	0.03667	-0.2821 to 0.3554	No	NS	0.9949
LPS vs. LPS+Doxy(0.1µg)	B-C	1.690	1.371 to 2.009	Yes	****	<0.0001
LPS vs. LPS+Doxy(0.5µg)	B-D	1.253	0.9346 to 1.572	Yes	****	<0.0001
LPS vs. LPS+Doxy(1µg)	B-E	0.8800	0.5612 to 1.199	Yes	****	<0.0001
LPS+Doxy(0.1µg) vs. LPS+Doxy(0.5µg)	C-D	-0.4367	-0.7554 to-0.1179	Yes	**	0.0078
LPS+Doxy(0.1µg) vs. LPS+Doxy(1µg)	C-E	-0.8100	-1.129 to-0.4912	Yes	****	<0.0001
LPS+Doxy(0.5µg) vs. LPS+Doxy(1µg)	D-E	-0.3733	-0.6921 to-0.05455	Yes	*	0.0209

LPS-Lipopolysaccharide, Doxy-Doxycycline hyclate

system focusses on discriminating between 'self and non-self', the system also functions to detect and protect against 'danger' signals.[10] Damage associated molecular patterns (DAMP's) encompass both exogenous microbe derived molecules and endogenous products expressed from damaged or dying cells. Alarmins are endogenous DAMP's[11] and have silent, physiologic and 'day time' functions within the cells and have immune functions extracellularly. S100 proteins along with high mobility group box 1 protein (HMGB1), heat shock proteins (HSP60, 70, Gp96) belong to the family of alarmins.[12] The S100's are a family of 25 calcium binding proteins. The S100 proteins are encoded by genes located on chromosome 1q21. S100A8, S100A9, S100A12 are myeloid related proteins[13] and are also called calgranulins.[14] S100A8 and S100A9 can exist as homodimers or preferentially as heterodimer S100A8/9 or calprotectin.[13] These proteins are increasingly being recognized for their prominent role in periodontal pathogenesis and for their potential as biomarkers of periodontal diseases.[15,16] S100A8 and S100A9 are immune modulators principally expressed by the neutrophils and to a lesser extent from the monocytes.[17] They are also expressed by inflamed keratinocytes, epithelial cells and osteoclasts.[13] Evidence points out that cells of the junctional epithelium express both S100A8 and S100A9.[18]



LPS-Lipopolysaccharide, Doxy-Doxycycline hyclate

Fig. 1: Cell viability

Doxycycline which is a semisynthetic tetracycline has been widely used in the management of oral infections for its antibacterial and host modulatory functions. In its standard adult oral dosage of 100-200mg/day it exerts antibacterial effects.[19] At lower concentrations of 20mg/twice daily doxycycline can inhibit matrix metalloproteinase activity[20] and modulate the release of inflammatory mediators from host cells.[21]

This in vitro study sought to find out if gene expression of S100A8 and S100A9 occurs from P. gingivalis LPS(Pg-LPS) induced HGF's. Next, the study explored the effects of doxycycline 0.1,0.5 and 1µg/mL on the gene expression of S100A8 and S100A9 from P. gingivalis induced HGF's.

Materials and Methods

Human gingival tissues were obtained from healthy individuals during crown lengthening, following informed

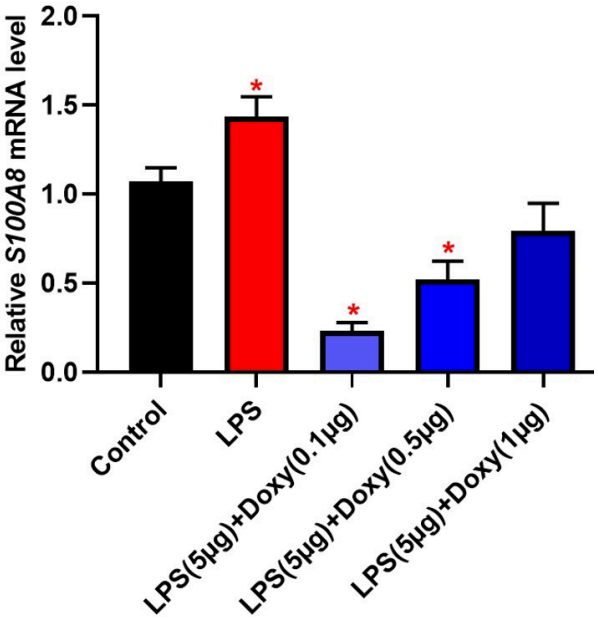


Fig. 2: S100A8 mRNA expression

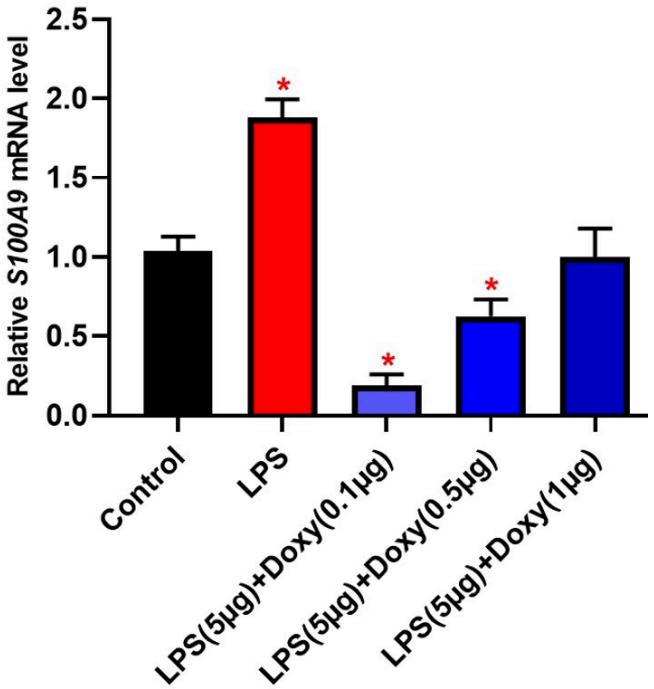


Fig. 3: S100A9 mRNA expression

consent, for the establishment of primary HGF cultures using a standardized protocol.[22] The study was conducted with prior approval from the Institutional Ethics Committee (IHEC/PhD/PERIO-1621/21/230) of Saveetha Dental College and Hospital. Collected gingival tissues were thoroughly rinsed with phosphate-buffered saline (PBS) supplemented with antibiotic–antimycotic agents to maintain sterility. The tissues were then processed to isolate and culture HGF's under controlled conditions. Upon reaching adequate confluence, the cells were stimulated with Pg-LPS- 5µg/mL (Sigma-Aldrich, St. Louis, MO, USA) for 24 hours in a CO_2 incubator. Subsequently, total RNA was extracted from both treated and control groups using standard extraction protocols.

RNA extraction and qPCR analysis: Total RNA was extracted from HGF cells using TRIzol reagent (Thermo Fisher Scientific, USA) according to the instructions from the manufacturer. Total RNA quantity and quality were assayed by using Nanodrop One (Thermo Scientific, USA). Total RNA was converted into complementary DNA (cDNA) using the Takara 1st strand cDNA synthesis kit (Takara, Tokyo, Japan), adhering strictly to the instruction manual. The mRNA expression levels of S100A8 and S100A9 were quantified by qPCR using the CFX 96 Real-Time PCR (Bio-Rad, Hercules, CA, USA) detection system following a standard SYBR Green PCR protocol using SYBR Premix Ex Taq™ (Takara, Japan). The list of primer sequences which were used for qPCR is listed in Table 1

Cell viability assay: The cytotoxic effects of doxycycline hyclate on HGF's were evaluated using the MTT assay prior to gene expression analysis. Cells were treated with varying concentrations of doxycycline (0.1, 0.5, and 1 µg/mL) following stimulation with Pg-LPS. After incubation, MTT solution was added and metabolically active cells reduced the tetrazolium salt into insoluble formazan crystals. The crystals were solubilized using dimethyl sulfoxide, and absorbance was measured spectrophotometrically at 570 nm. Cell viability was expressed as a percentage relative to untreated control cells. Concentrations demonstrating >80–90% viability were considered non-cytotoxic and selected for subsequent gene expression analysis.

Subsequently, doxycycline in concentrations of 0.1, 0.5 and 1µg/mL was added to the gingival fibroblasts, which were treated with Pg-LPS for 24 hours, and RNA extraction and quantification were done after another 24 hours. The study groups were-Group A: Relative gene expression in Control, Group B: Relative gene expression after Pg-LPS stimulation, Group C: Relative gene expression after Pg-LPS stimulation and addition of 0.1µg/mL doxycycline, Group D: Relative gene expression after Pg-LPS stimulation and addition of 0.5µg/mL doxycycline and Group E: Relative gene expression after Pg-LPS stimulation and addition of 1µg/mL doxycycline. The reaction conditions consisted of an initial denaturation at 95 °C for 3 min, followed by cycles of 95 °C for 10 s and 58 °C for 30 s (repeated for 39 cycles). The GAPDH gene was used as a housekeeping gene. The $2^{-\Delta\Delta Ct}$ method was utilized to estimate relative mRNA levels and experiments were performed in triplicate.

Statistical analysis: Data was entered into spread sheets and analyzed using SPSS 24. Comparison of means in the groups was done by one way ANOVA. Intergroup differences were compared using Tukey's multiple comparison test. A p-value < 0.05 was considered to be statistically significant.

Results

On stimulation of HGF's with Pg-LPS, mean difference in fold change of mRNA expression of S100A8 was 0.3633 when compared to the control group and the difference between the control group A and the LPS group B was statistically significant (p=0.0112). For S100A9, the mean difference in fold change of mRNA expression between groups A and B was 0.8433 which was highly significant. (p<0.0001)

Treatment with doxycycline at concentrations of 0.1, 0.5, and 1 µg/mL did not significantly reduce the viability of LPS-stimulated HGF's compared with control cells (p>0.05). Cell viability remained above 85% at all tested concentrations, indicating absence of cytotoxic effects. These concentrations were therefore used for subsequent evaluation of S100A8 and S100A9 gene expression. Figure 1 depicts results of MTT assay.

Gene expression of S100A8 decreased significantly on addition of doxycycline to the cell cultures. The mean difference in mRNA expression was 1.203 (p<0.0001), 0.913 (p<0.0001) and 0.643 (p=0.0001) when doxycycline in concentrations of 0.1, 0.5 and 1µg/mL respectively were added to gingival fibroblast cultures induced by Pg-LPS. Gene expression of S100A9 also decreased significantly on addition of doxycycline. The mean difference in mRNA expression was 1.690 (p<0.0001), 1.253 (p<0.0001) and 0.880 (p<0.0001) when doxycycline in concentrations of 0.1, 0.5 and 1µg/mL respectively were added to Pg-LPS induced HGF's. Figure 2 depicts the gene expression of S100A8 from Pg-LPS induced HGF's and the effects of 0.1, 0.5 and 1µg/mL doxycycline on the same. Figure 3 represents the gene expression of S100A9 from Pg-LPS induced HGF's and the effects of the different concentrations of doxycycline on the mRNA expression profile. Results of the experiment are summarized in Table 2 and 3.

Discussion

This study investigated the gene expression of S100A8 and S100A9 from Pg-LPS induced HGF's. In vitro gene expression assay pointed out that both S100A8 and S100A9 are significantly expressed by gingival fibroblasts when stimulated by LPS. Addition of doxycycline in concentrations of 0.1,0.5 and 1µg/mL caused decrease in the gene expression of both these mRNAs. The maximum decrease was seen at 0.1µg/mL.

In this study, an in vitro inflammation model was generated using a concentration of 5µg/ml of Pg-LPS. P. ginivalis is now recognized as the 'keystone pathogen' in dysbiotic biofilms and is considered the initiating factor in periodontal inflammation. This facultative gram negative, even in small numbers can cause perturbances in the plaque biofilm. They persist in the anaerobic environment of periodontal pockets and express cysteine proteinases that dysregulate host innate immune responses in order to persist and survive in the periodontal tissues.[7] The LPS is present in its outer cell membrane and is composed of the core, lipid A and O-specific polysaccharide. The LPS structure of P. gingivalis has distinct tetra and penta

acetylations and activate diverse signaling pathways.[23]

Adding on to the evidence that gingival fibroblasts express an array of inflammatory mediators,[24] this in vitro study provides pilot evidence for expression of both S100A8 and S100A9 from Pg-LPS induced HGF's.

Doxycycline which is a semisynthetic tetracycline has antibacterial and anti-inflammatory actions. The anti-inflammatory action is primarily due to its ability to inhibit matrix metalloproteinase (MMP) activity.[21] It is widely used in the management of periodontal diseases due its ability to concentrate in gingival crevicular fluid and 20 mg of Doxycycline or Periostat is prescribed as a host modulatory agent.

The MTT assay confirmed that doxycycline concentrations used in the present study did not exert cytotoxic effects on HGF's. Therefore, the observed modulation in S100A8 and S100A9 gene expression can be attributed to the anti-inflammatory properties of doxycycline rather than reduced cellular viability. In this study doxycycline was used to explore the anti-inflammatory action of this drug on the gene expression of S100A8 and S100A9 from LPS induced HGF's. As the mRNA expression of both these genes decreases with the addition of doxycycline, it may be deduced that both these genes are proinflammatory. The proinflammatory nature of these genes by virtue of its neutrophilic chemotactic action was also pointed out in previous studies.[25] Evidence points out that in primary epithelial and oral cancer cells, Pg-LPS binds to TLR's to initiate NFκB/MAPK signaling pathways resulting in pro inflammatory gene expression.[26] TLR signalling involves myeloid differentiation primary-response protein kinases 88(MyD88), leading to the activation of transforming growth factor-β-activated kinase 1 (TAK1) which induce NF-kB, c-Jun N-terminal kinase (JNK) and p38 pathways.[26] Periodontal fibroblasts also respond actively to Pg-LPS stimulation via MAPK pathways such as ERK and p38 leading to production of pro-inflammatory mediators such as IL-8 and IL-6.[27]

To the best of our knowledge, this study is the first report on the expression of both S100A8 and S100A9 from LPS induced HGF's. The study also underlines the host modulatory role of doxycycline. The study is limited by the fact that it examined only the expression of the two S100 proteins from LPS induced HGF's and did not compare the mRNA expression of S100's to that of prominent proinflammatory cytokine such as IL-1β.

Conclusion

This in vitro model of periodontal inflammation demonstrates that HGF's significantly express S100A8 and S100A9 genes when exposed to Pg-LPS. Additionally, low doses of doxycycline can inhibit the mRNA expression of these genes. Gingival fibroblasts may be considered as potential targets for host modulatory therapy.

References

1. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34. doi:10.1111/prd.12331
2. Tattar R, da Costa BDC, Neves VCM. The interrelationship between periodontal disease and systemic health. Br Dent J. 2025;239(2):103-108. doi:10.1038/s41415-025-8642-2
3. Wielento A, Lagosz-Cwik KB, Potempa J, Grabiec AM. The Role of Gingival Fibroblasts in the Pathogenesis of Periodontitis. J Dent Res. 2023;102(5):489-496. doi:10.1177/00220345231151921
4. Jang JY, Song I -S., Baek KJ, Choi Y, Ji S. Immunologic characteristics of human gingival fibroblasts in response to oral bacteria. J Periodontal Res. 2017;52(3):447-457. doi:10.1111/jre.12410
5. Mahanonda R, Sa-Ard-Iam N, Montreekachon P, et al. IL-8 and IDO Expression by Human Gingival Fibroblasts via TLRs. J Immunol. 2007;178(2):1151-1157. doi:10.4049/jimmunol.178.2.1151
6. Schuster A, Nieboga E, Kantorowicz M, et al. Gingival fibroblast activation by Porphyromonas gingivalis is driven by TLR2 and is independent of the LPS-TLR4 axis. Eur J Immunol. 2024;54(3). doi:10.1002/eji.202350776
7. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10(10):717-725. doi:10.1038/nrmicro2873
8. Hajishengallis G, Diaz PI. Porphyromonas gingivalis: Immune Subversion Activities and Role in Periodontal Dysbiosis. Curr Oral Heal Reports. 2020;7(1):12-21. doi:10.1007/s40496-020-00249-3
9. Marchesan JT, Girnary MS, Moss K, et al. Role of inflammasomes in the pathogenesis of periodontal disease and therapeutics. Periodontol 2000. 2020;82(1):93-114. doi:10.1111/prd.12269
10. Matzinger P. Tolerance, Danger, and the Extended Family. Annu Rev Immunol. 1994;12(1):991-1045. doi:10.1146/annurev.iy.12.040194.005015
11. Yang D, Han Z, Oppenheim JJ. Alarmins and immunity. Immunol Rev. 2017;280(1):41-56. doi:10.1111/imr.12577
12. Holzinger D, Tenbrock K, Roth J. Alarmins of the S100-Family in Juvenile Autoimmune and Auto-Inflammatory Diseases. Front Immunol. 2019;10. doi:10.3389/fimmu.2019.00182
13. Xia P, Ji X, Yan L, Lian S, Chen Z, Luo Y. Roles of S100A8, S100A9 and S100A12 in infection, inflammation and immunity. Immunology. 2024;171(3):365-376. doi:10.1111/imm.13722
14. Gonzalez LL, Garrie K, Turner MD. Role of S100 proteins in health and disease. Biochim Biophys Acta - Mol Cell Res. 2020;1867(6):118677. doi:10.1016/j.bbamcr.2020.118677
15. Holmström SB, Lira-Junior R, Zwicker S, et al. MMP-12 and S100s in saliva reflect different aspects of periodontal inflammation. Cytokine. 2019;113:155-161. doi:10.1016/j.cyto.2018.06.036
16. Lira-Junior R, Bissett SM, Preshaw PM, Taylor JJ, Boström EA. Levels of myeloid-related proteins in saliva for screening and monitoring of periodontal disease. J Clin Periodontol. 2021;48(11):1430-1440. doi:10.1111/jcpe.13534
17. Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in Inflammation. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.01298
18. Nishii K, Usui M, Yamamoto G, et al. The distribution and expression of 100A 8 and S100 A 9 in gingival epithelium of mice. J Periodontal Res. 2013;48(2):235-242. doi:10.1111/jre.12000
19. Golub LM, Elburki MS, Walker C, et al. Non-antibacterial tetracycline formulations: host-modulators in the treatment of periodontitis and relevant systemic diseases. Int Dent J. 2016;66(3):127-135. doi:10.1111/idj.12221
20. Golub LM, Lee H. Periodontal therapeutics: Current host-modulation agents and future directions. Periodontol 2000. 2020;82(1):186-204. doi:10.1111/prd.12315
21. Cazalis J, Bodet C, Gagnon G, Grenier D. Doxycycline Reduces Lipopolysaccharide-Induced Inflammatory Mediator Secretion in Macrophage and Ex Vivo Human Whole Blood Models. J Periodontol. 2008;79(9):1762-1768. doi:10.1902/jop.2008.080051
22. Diar-Bakirly S, El-Bialy T. Human gingival fibroblasts: Isolation, characterization, and evaluation of CD146 expression. Saudi J Biol Sci. 2021;28(4):2518-2526. doi:10.1016/j.sjbs.2021.01.053

23. Aleksijević LH, Aleksijević M, Škrlec I, Šram M, Šram M, Talapko J. Porphyromonas gingivalis Virulence Factors and Clinical Significance in Periodontal Disease and Coronary Artery Diseases. Pathogens. 2022;11(10):1173. doi:10.3390/pathogens11101173
24. Xu S, Zhou Q, Fan C, et al. Doxycycline inhibits NAcht Leucine-rich repeat Protein 3 inflammasome activation and interleukin-1β production induced by Porphyromonas gingivalis-lipopolysaccharide and adenosine triphosphate in human gingival fibroblasts. Arch Oral Biol. 2019;107:104514. doi:10.1016/j.archoralbio.2019.104514
25. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory Activities of S100: Proteins S100A8, S100A9, and S100A8/A9 Induce Neutrophil Chemotaxis and Adhesion. J Immunol. 2003;170(6):3233-3242. doi:10.4049/jimmunol.170.6.3233
26. Groeger S, Jarzina F, Domann E, Meyle J. Porphyromonas gingivalis activates NFκB and MAPK pathways in human oral epithelial cells. BMC Immunol. 2017;18(1):1. doi:10.1186/s12865-016-0185-5
27. Seo T, Cha S, Kim TI, Lee JS, Woo KM. Porphyromonas gingivalis-derived lipopolysaccharide-mediated activation of MAPK signaling regulates inflammatory response and differentiation in human periodontal ligament fibroblasts. J Microbiol. 2012;50(2):311-319. doi:10.1007/s12275-012-2146-x