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Review article

Integrative analysis of gene and protein expression in atherosclerosis-related pathways modulated by periodontal pathogens. Systematic review☆

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ABSTRACT

The mechanisms modulated by periodontal pathogens in atherosclerosis are not fully understood. Aim: to perform an integrative analysis of gene and protein expression modulated by periodontal pathogens in cells and animal models for atherosclerosis.

Methods: Cochrane, PRISMA and AMSTAR2 guidelines for systematic reviews were followed. Data search was conducted in Pub-med, LILACS and Science Direct databases. Gene and protein expression data were collected from the included papers to perform an overrepresentation analysis using the Reactome Pathway Analysis tool and the KEGG database.

Results: Thirty-two papers were included in the review, they analyzed the effect of *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus anginosus*, *Streptococcus sanguinis*, *Tannerella forsythia*, and *Treponema denticola* or/and their virulent factors on gene and protein expression in human cells and animal models of atherosclerosis. Some of the modulated pathways include the immune system, programmed cell death, cellular responses to external stimuli, transport of small molecules, and signal transduction ($p < 0.05$). Those pathways are known to be involved in different stages of atherosclerosis progression.

Conclusion: Based on the performed analysis, it is possible to state that periodontal pathogens have the potential to be a contributing factor for atherosclerosis even in absence of a high-fat diet or high shear stress.

Contents

☆ Scientific field of dental biology: Molecular and cell biology.

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1. Introduction

Periodontitis and atherosclerosis are chronic inflammatory diseases [1]. Cardiovascular diseases, including atherosclerosis, are the leading cause of death worldwide, taking 17.9 million lives per year [2]. Endothelial dysfunction, high serum lipoprotein concentration and lipid accumulation in the inner walls of large arteries, are crucial for the initiation of atherosclerosis [3]. By its side, periodontitis is the main cause of tooth loss. Its prevalence worldwide ranges from 20% to 50%. This disease presents an upward trend among individuals older than 35 years [4]. The dysbiosis in the oral microflora is the main causative agent of periodontitis [5]. An association between periodontitis and cardiovascular events such as stroke, myocardial infarction, abdominal aortic aneurysm and cardiovascular death has been observed [6–9].

It has been stated that periodontitis could be a contributing factor for atherosclerosis [10], not only by the increase of the systemic inflammatory burden but also by transitory bacteremia present in patients that suffer from this disease [11]. Several studies have identified periodontal pathogens in atherosclerotic plaques but the effect of those microorganisms in the initiation and progression of atherosclerosis is not completely understood [12,13].

To clarify the link between periodontitis and atherosclerosis, different studies have applied cell and molecular biology methods, to detect changes in gene and protein expression when animal and human cell models are exposed to periodontal pathogens or their virulence factors. Nevertheless, their results have not been globally analyzed. An integrative analysis of the results of these studies, based on pathways overrepresentation analysis, is necessary to facilitate a comprehensive understanding of the effect of the periodontal pathogens on different pathways involved in atherosclerosis.

Bioinformatics tool can be used to integrate the results of transcriptomic and proteomic studies to identify pathways modulated under certain conditions. Different tools can be used to perform this integrative analysis. Reactome.org (Reactome) is a database that links human genes, proteins and other entities participating in reactions into networks, that are grouped into pathways. Those pathways are then grouped into superpathways or nodes [14]. Another useful database to performed this kind of analysis is the Kyoto Encyclopedia of Genes and Genomes pathway annotation database (KEEG), that permits the overlapping of genes to specific disease pathways [15].

Therefore, this systematic review was aimed to perform an integrative analysis of the reported genes and proteins modulated by periodontal pathogens in cells and animal models for atherosclerosis. The research question of this systematic review was, what is the effect of periodontal pathogens on the pathways involved in atherosclerosis? The results of this study shows that periodontal pathogens are a potential contributing factor for atherosclerosis even in absence of a high-fat diet or high shear stress.

2. Materials and methods

Study design and methodology were defined before conducting the literature search, and were based on The Cochrane handbook guidelines [16], the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [17] and the AMSTAR 2 tool for systematic reviews [18].

2.1. Selection criteria

(a) Primary studies, reporting the use of animal and human models, (b) evaluation of the role of periodontal pathogens on atherosclerosis, (c) published papers or in-press that reported peer-review assessment, (d) papers published in English, Spanish, or French, (e) in-vitro and in-vivo studies, (f) papers answering the research question of this systematic review, (g) abstract mentioning the use of cell and molecular biology methods. In cases where the abstract omitted information about materials and methods, the paper was included for in-full text reading.

2.2. Exclusion criteria

(a) Papers studying the role of periodontal pathogens on other systemic diseases but not atherosclerosis, (b) use of antibiotics or any other treatment for periodontitis or atherosclerosis, (c) apical periodontitis studies, (d) association studies, (e) risk factor studies for either periodontitis or atherosclerosis.

2.3. Information sources and search

The following electronic databases were systematically searched: Pub-med, LILACS and Science Direct, including papers published from January 1st 2015 to August 31st 2020. To collect the most recent available evidence.

2.4. Search strategy

After defining the objective of this systematic review, the research strategy was based on the research question and a search equation. The equation was constructed combining Medical Subject Headings (MeSH) and free text words, using the following keywords: periodontal pathogens, periodontitis, molecular mechanisms, endothelial dysfunction and atherosclerosis.

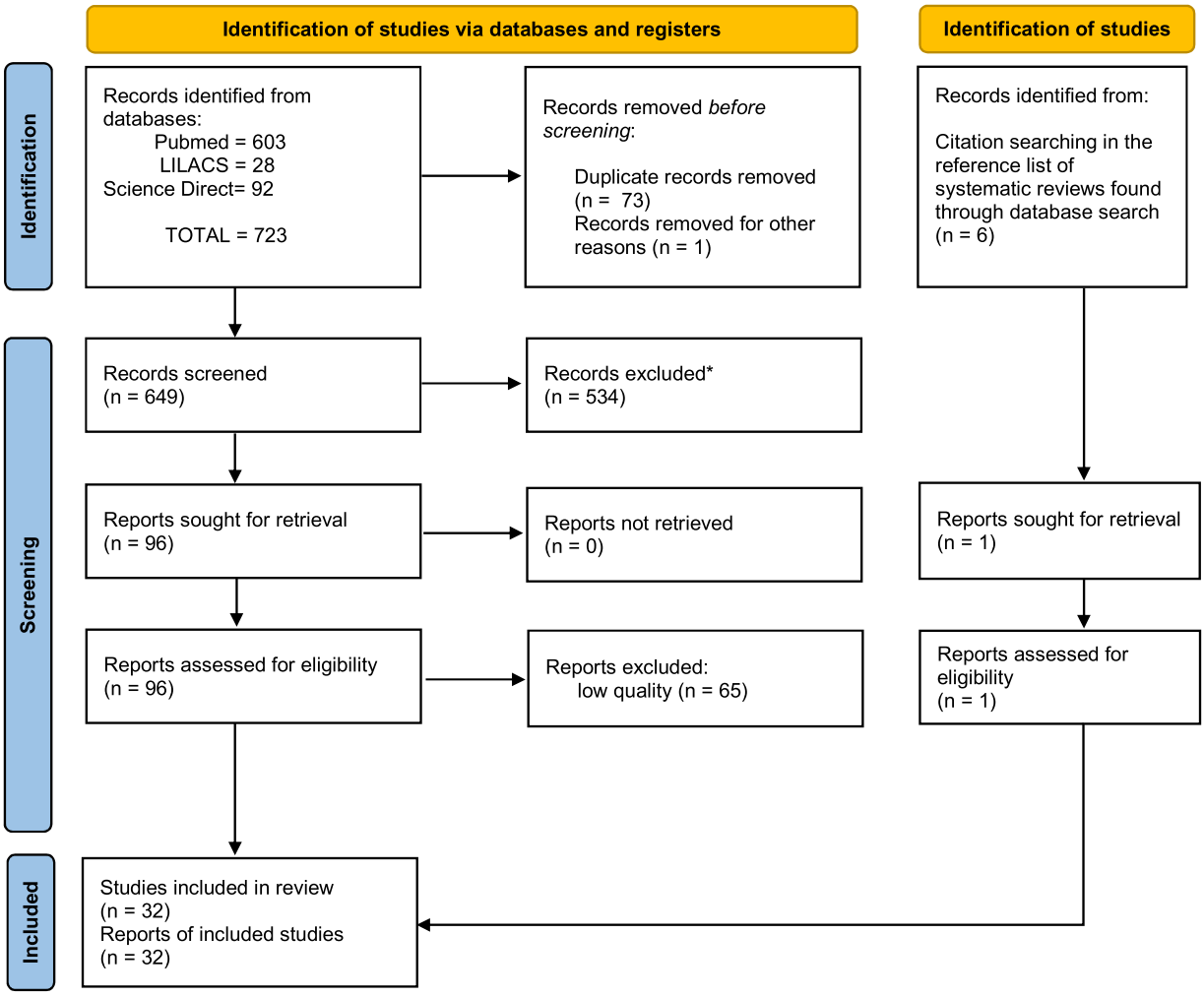


Fig. 1. Prisma flow diagram 2020.

The search was performed in the databases afore listed, using the following search equation: (periodontitis OR "periodontal disease") AND (atherosclerosis OR "cardiovascular diseases" OR "coronary artery" OR "coronary artery disease"). Once the search equation was defined, the first observer (TYM) applied it in the databases and the second observer (SGO) confirmed the results. Snowball-search strategies were also applied to find additional relevant studies among the systematic reviews discovered in the first search.

2.5. Study selection

After duplicates deletion, title and abstract analysis of the collected studies were independently performed by the first observer to select records for in-full text reading. The second observer independently checked the non-included records to confirm their exclusion. Any disagreement was solved through consensus.

2.6. Data collection process

Data extraction was performed by TYM and checked by SGO. A data collection tool was designed in an Excel spreadsheet. This tool was applied to studies that matched the inclusion criteria and to those studies that omitted information about the methodology in the abstract. Excluded papers after full-text evaluation were recorded in a table with the respective exclusion reason.

2.7. Data items

Data extracted in Excel included: study ID (authors, year of publication, country), study design, methodology, the subject of study (animal aorta and cardiac tissue, cells, blood and human cells), the intervention (bacteria strain or virulence factor), variables to analyze (gene expression, protein expression, clinical, cell, or histological changes), methods, outcome understood as the change in gene and/or protein expression (decrease, increase, no change), significance level and the clinical or cellular events induced by different bacteria strains or virulence factors. To perform the pathways overrepresentation analysis, gene and protein names were unified using the uniprot.org [19], NCBI.gene [20] and PANTHER [21] databases.

2.8. Quality assessment

The tools for quality assessment STROBE [22] and CONSORT 2010 [23] were adapted to assess the quality of the retrieved papers. A total of 14 items were evaluated per paper, giving a score of 1 (met the criteria) or 0 (did not meet the criteria).

This procedure provided a total score that ranged from 0 to 14. An ordinal quality scale was set: low ≤ 8, medium 9–12 and high ≥ 13. This scale was determined to ensure that the included papers met more than 60% of the evaluated items. Papers with low quality were not included in the analysis.

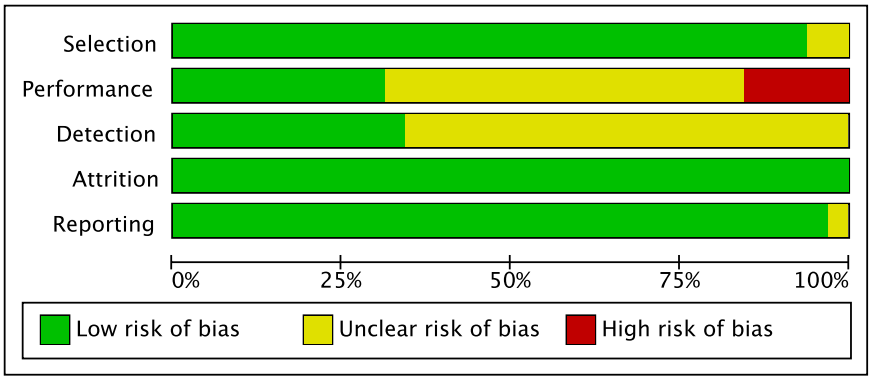


Fig. 2. Risk of bias assessment.

2.9. Risk of bias assessment

The risk of bias was assessed using the Revman 5.4 software [24]. Criteria were established following and adapting the approach to address in-vitro studies proposed by the Office of Health Assessment and Translation (OHAT) [25,26] and the Cochrane guidelines [16].

Selection (concealed allocation to study groups), performance (experimental conditions and blinding of operators), detection (reliability on outcome assessment), attrition (data competition and integrity) and reporting (selective or unclear results reporting) bias were evaluated.

2.10. Statistical analysis

The genes and proteins which expression was modulated by periodontal pathogens were included in the overrepresentation analysis in the Reactome Pathway-Analysis tool (up or down-regulated with $p < 0.05$) [14]. This analysis was performed separately for each human cell type and animal species. For this analysis, a False Discovery Rate (FDR) of 5% was used to control the rate of false positives. The same genes and proteins were fed into KEGG to visualize the effect of the periodontal pathogens on the lipids and atherosclerosis pathway, (hsa05417) and the fluid shear and atherosclerosis pathway (hsa05418) in human cells and mice [27].

3. Results

3.1. Quality and risk of bias assessment

Data search produced a total of 722 papers. The studies selected for full-text reading (n = 97, 96 from database search and one from snowball search) were ranked as low (n = 65), medium (n = 23) and high quality (n = 9) according to the established criteria. Papers with low quality were excluded (n = 65) and 32 papers remained (Fig. 1, Supplementary Table 1) for data collection (Supplementary Table 2). Fig. 2 and Supplementary Figure 1 show the risk of bias assessment for these 32 papers.

3.2. Subjects of the study and exposure

The subjects of the studies were human cells, mice and rabbits. The 32 included studies reported the effects of different periodontal pathogens on gene or protein expression in different cells and tissues including, Human Aortic Endothelial Cells (HAECS), human Aortic Smooth Muscle Cells (ASMCs), Human Umbilical Vein Endothelial Cells (HUVEC), Human umbilical vein cell line EA.hy926 (EA.hy926), Human Monocytic Cell Line THP-1, CD14 + monocytes, mice serum/blood, mice aortic tissue, mice macrophages and rabbit sera. The models were exposed to different periodontal pathogens. The bacteria species used were *Eikenella corrodens, Filifactor alocis, Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus anginosus, Streptococcus sanguinis, Tannerella forsythia* and *Treponema denticola*, or their virulence factors: lipopolysaccharide (LPS), outer membrane vesicles (OMV) and gingipains.

3.3. Gene expression and pathway overrepresentation analysis (Reactome)

For human cells, a total of 87 genes and 59 proteins were analyzed. Forty-eight genes and 49 proteins showed significant changes in their expression after exposure to periodontal pathogens or their virulence factors (Table 1).

Seventy-nine proteins and genes were upregulated and 18 were downregulated. In mice, 119 genes and 73 proteins were analyzed. The expression of 55 genes and 55 proteins showed significant changes after exposure to periodontal pathogens. Eighty-three genes and proteins were upregulated and 13 were downregulated (Table 2). In rabbits, 6 genes and 7 proteins were assessed, all of them were upregulated (Table 2).

Fig. 3 presents a diagram of pathways modulated by periodontal pathogens in human cells according to the overrepresentation analysis in Reactome (FDR < 0.05). This analysis reveals an important activation of superpathways that participate in the establishment and progress of atherosclerosis, such as the “immune system”, “programmed cell death”, “cellular responses to external stimuli” and “transport of small molecules”.

The “signal transduction” and the “homeostasis” superpathways were connected through the activation of the “Integrin and the platelet aggregation” pathway. The “immune system” superpathway includes “MAP kinase activation”, “innate immune system”, “NLRP3 inflammasome”, “interleukins (IL)” and “toll-like receptor (TLR) cascades” pathways that are present in the continuous inflammatory response promoting the development of atherosclerosis.

Fig. 4 presents the top 25 overrepresented pathways in humans. Three of the modulated pathways were shared by HAECs, HUVECs, ASMCs and THP-1: the “Diseases associated with the TLR signaling cascade”, “Interleukin-4 and Interleukin-13 signaling” and “MyD88:MAL(TIRAP) cascade initiated on plasma membrane” pathways. The analysis for CD14 + cells was not included as the expression of only five proteins was reported, IL-10, IL-1β, IL-6, IL-8 (CXCL8) and tumor necrosis factor-alpha (TNF-α). All of them showed a significant increase in their expression when the cells were exposed to *P. gingivalis*.

3.4. Analysis of KEGG atherosclerosis pathways

KEGG is a database that facilitates the understanding of high-level functions, from molecular-level information [27]. The data

Table 1
Genes and proteins with significantly different expression in human cells or tissues after treatment with periodontal pathogens or their virulence factors. (Genes in italics, proteins in script).

Author, Year	Cell type	Bacteria	Strain/virulence factor	Method	Upregulated gene or protein	Downregulated gene or protein
Hirasawa & Kurita-Ochiai., 2018 [40]	HUVECS	*P. gingivalis*	Strain FDC381	Caspase Assay, rt-PCR, sD PAGE, western blot	*BECN1*, CASPASE1, CASPASE12, CASPASE3, CASPASE8, CASPASE9, DDIT3, *DDIT3*, HSPA5, *HSPA5*, MAP1LC3A, MAP1LC3B, *MAP1LC3B*	
	HUVECS	*P. gingivalis*	Strain KDP136	Caspase Assay, rT-PCR, sD PAGE, western blot		
Huang et al., 2016 [45]	HAECS THP-1	*P. gingivalis*	Groel	rt-PCR, western blot	ELAVL1, HNRNPD, OLR1, TLR4, *TLR4*, ZFP36 ICAM1, SELE, VCAM1	TLR2
Jayaprakash et al., 2017 [49]	THP-1	*P. gingivalis*	Strains: Pg w50, PgE8, PgK1a	rt-PCR western Blot	*CASPASE1, CXCL8, F2RL1, MARK2, NOD1, NOD2, PARD3, TLR1, TLR4, TLR6, TLR9*	
			Strains: Pg E8, PgK1A, Pg W50 + inhibition of p38, PK,NFkB,ERK			*IL-1β*
			Strain W50		*TLR2*	
Kim et al., 2018 [63]	THP-1	*P. gingivalis*	Strain ATCC33277	Gelatin zymography ELISA	MMP9, *MMP9*, TNF	
Køllgaard et al., 2017 [64]	CD4 +	*P. gingivalis*	LPS Arginine-gingipain	Flow cytometry	IL-1β, IL-10, IL-6, CXCR1, TNF	
Li et al., 2020 [47]	HUVECS	*P. gingivalis*	Strain W83	rt-PCR Western Blot	CCL2, *CCL2*, ICAM1, *ICAM1*, LOX1, *LOX1*, RELA, *RELA*, SELE, *SELE*	
	THP-1				CCR2, *CCR2*, ITGA2B, *ITGA2B*, ITGB2, *ITGB2*, OLR1, *OLR1*, RELA, *RELA*.	
Liu et al., 2016 [43]	THP-1	*P. gingivalis*	LPS	rt-PCR, Western blot	ACAT1, *ACAT1*	ABCG1, *ABCG1*, SCARB1
Suh et al., 2019 [32]	HUVECS	*P. gingivalis*	LPS	ELISA, immunofluorescence, western blot	*AIFM2, GATA4, PECAM1, SNAI2, TWIST1* AIMF, ICAM1, PECAM1, TWIST1, VCAM1	
	THP-1		LPS		IL-1β, TNF, IL-6	
Viafara-Garcia et al., 2018 [50]	HAECS	*E. coli*	LPS ATCC 0111:B4	ELISA, Flow cytometry, western blot	CSF2, CXCR1, ICAM1	CCL5
		E. corrodents	LPS		CCL2, CSF2, CXCR1, ICAM1, MAPK3/MAPK2	
			LPS+Anti TLR4			CCL2, CSF2, CXCR1
Wan et al., 2015 [60]	HUVECS	*P. gingivalis*	Sonicates	PCR, rt-PCR, western blot	*NOD1, NOD2*, SELE, TLR2	
			Strain W83		NOD1 NOD2 RELA SELE TLR1 TLR2	
			Strain WT		RELA	MAPK1, RELA, *SELE*, SELE (in transfected cells with siRNA NOD2 or with siRNA TLR2)
Wang et al., 2020 [39]	HUVECS	*P. gingivalis*	LPS	ELISA, qrt-PCR, rt-PCR, western blot	AKT1, CCL2, *CCL2*, CXCR1, *CXCR1*, ICAM1, *ICAM1*, RELA, SELE	AKT1 (gas6 KO mice), GAS6, RELA(gas6 KO mice), *SELE*
Wu et al., 2019 [67]	EA.hy926	*P. gingivalis*	Strain ATCC 33277	western blot, qRT-PCR	CXCR4, ICAM1, *ICAM1*	
Xie et al., 2020 [13]	HAECS	*P. gingivalis*	Strain W38	ELISA, qrt-PCR, rt-PCR, western blot, flow cytometry	BAX, CASPASE3, IL-1β, IL-6, IRAK2, NFKBIA, RELB, TNF, TRAF1, *ICAM1, SELE, VCAM1*	ARNTL, BCL2, *CD34, Clock, CRY1, CRY2, NR1D1, NR1D2, PER1, PER2, PER3*
	HASMCs					ARNTL, *ARNTL*
Xu et al., 2018 [46]	EA.hy926	*P. gingivalis*	Strain ATCC33277	ELISA, qRT-PCR, western blot	ICAM1, *ICAM*, MIF	
Zhang et al., 2015 [44]	AoSMCs	*P. gingivalis*	Starains: WT ATCC 33277, WT 381,W50, E8, K1A, fimbira mutans DPG3, fimbria mutans KRX178, P.g LPS,	MTT assay, rt-PCR, western blot	ANGPT2, *ANGPT2, ETS1*	*ANGPT1*
		F. alocis	Strain ATCC35896		ANGPT2	
Zhang et al., 2016 [71]	AoSMCs	*P. gingivalis*	Strains: Pg w50, PgE8, PgK1a	Disease ontology analysis qRT-PCR, rt-PCR	*ADA, AKR1B10, ANGPT2, APOC1, CCL11, Cx3cl1, CXCL8, EDN1, EDNRA, GJA4, IGFBP1, IL-1A, IL-7, ITGA2, ITGB3, KALRN, LIPG, MCAM, NOD1, PDGFD, PLAT, PLAU, RAMP1, RASL11B, SGPP1, SLC2A9, TLR1*	

Table 2
Genes and proteins with significantly different expression in animal tissues after treatment with periodontal pathogens or their virulence factors. (Genes in italics, proteins in script).

Author Year	Specie	Subject model	Bacteria/ Strain/virulence factor	Methods	Tissue	Upregulated gene or protein	Downregulated gene or protein
Brown et al., 2015 [34]	Mouse	$Cd36^{-/-}$/$Ldlr^{-/-}$, $Ldlr^{-/-}$ $Cd36^{-/-}$, WT, $TLR2^{-/-}$	*P. gingivalis* LPS	ELISA	Serum Macrophages	INFG, IL-6 IL-1β	
Chukkapalli et al., 2015 [10]	Mouse	hyperlipidemic ApoEnull mice (B6.129P2-ApoEtm1Unc/J)	*T. forsithya* ATCC 43037	Cytokine array, ELISA, rt-PCR	Aorta Serum	*BIRC3, IL-1A, IL-5, ITGA5, PPARA, RXRA, SERPINB2, SERPINE1* CCL2, CCL25, CCL5, CSF2, CX3CL1, FAS, IGG, IGM, IL-10, IL-12 β, IL-12p40/p70, IL-17RB, IL-1β, IL-3, IL-4, IL-9, LEP, TIMP1, TIMP2, TNF, TNFRSF8, XCL1, IGG, IGM	*APOA1, APOB, BCL2A1, CCL5, FGA, FGB, IL-1β, NYP, SELE* AKR1B10C, CL11, CXCL11, CXCL13, IL-6
Chukkapalli et al., 2015 [70]	Mouse	$ApoE^{-/-}$ B6.129P2-Apoetm1Unc/J mice	*P. gingivalis* ATCC 53977, *T. denticola* ATCC 35404, *T. forsythia* ATCC 43037, *F. nucleatum* 49256	ELISA, qRT-PCR, cytokine array	Serum	*NPY*, CCL5, CSF2, IFNG, IGG, IGM, IL-1β, IL-3, IL-4, XCL1, IGG, IGM, CCL5, CSF2, IFNG, IL-1β, IL-3, IL-4, XCL1	*APOA1, APOB, BIRC3, FGA, FGB*, CSF1, CXCL11, CXCL12, FAS, IL-13
Chukkapalli et al., 2017 [29]	Mouse	$TLR2^{-/-}$ $TLR4^{-/-}$	*P. gingivalis* ATCC 53977, *T denticola* ATCC 35404, *T. forsythia* ATCC 43037, *F. nucleatum* ATCC 49256	Cytokine array,ELISA, PCR, qPCR, rt-PCR,	Serum	CCL2 *HSPDD1, IL-2, JUN*	 *IL-6*
Chukkapalli et al., 2018 [31]	Mouse	$TLR2^{+/-}TLR4^{-/-}$ $TLR2^{-/-}TLR4^{+/-}$ $TLR2^{-/-}TLR4^{-/-}$	*P. gingivalis* ATCC 53977, *T. denticola* ATCC 35404, *T. forsythia* ATCC 43037, *F. nucleatum* ATCC 49256	ELISA	Blood	IGG	
Gupta et al., 2019 [41]	Mouse	WT, $TRPV4^{-/-}$	*P. gingivalis* LPS	Immunoblot, qRT-PCR	MRMs	*IL-1β, IL-6, TNF, TRPV4*	
Hashizume-Takizawa et al., 2019 [37]	Mouse	$APOE^{-/-}$	*P. gingivalis* ATCC 33277, *S. sanguinis* ATCC 10556, *S. anginosus* ATCC 12395	Cytokine array, rt-PCR, western blot	Aorta Serum	*IL-1A, MIR146A, TLR4, TNF, TRAF* *IL-1A*, CCL11, CSF1, FGF2	
Huang et al., 2016 [45]	Mouse	C57BL/6	*P. gingivalis* ATCC 33277 Groel	Immunohistochemistry	Aorta	CD68, ICAM1, OLR1, VCAM1	
Kurita-Ochiai., et al. 2917 [1]	Mouse	$ApoE^{-/-}$	*P. gingivalis* FDC381, *P. ginfivalis* LPS	Immunohistochemistry, rt-PCR	Aorta	*CYBB, DECR1, NCF1, NOD1, LOX1, TLR2, TLR4,* MPO	
Lin et al., 2015 [35]	Rabbit	Newzealand rabbits	*P. gingivalis*	ELISA, rt-PCR, western blot	Peripheral blood	*CCL2, CRP, IL-6, MMP9, TLR4,* CCL2, CRP, IL-6, MAPK1, NFKβ1, TLR2, TNF	
Lin et al., 2015 [61]	Rabbit	Newzealand rabbits	*P. gingivalis* LPS	Biochemical analysis, ELISA, rt-PCR, western *blot*	Peripheral blood	*CCL2,* CRP, *IL-6, MMP9*, NFKβ1, *TLR4, TNF,* CCL2, *CRP,* IL-6, NFKβ1, TLR2, TNF	
Liu et al., 2016 [48]	Rat	Wistar rats	*P. gingivalis* LPS	Rt-PCR	VSMCs	*ALPP, RUNX2, SPP1, IBSP*	
Suh et al., 2019 [32]	Mouse	Male $ApoE^{-/-}$	*P. gingivalis* LPS	immunofluorescence, western blot, ELISA,	Serum Aorta Atherosclerotic plaque	CRP, IL-1β, IL-6, TNF PECAM1, SNAI1 ICAM1, VCAM1	
Velsko et al., 2015 [28]	Mouse	WT, $ItgB6^{-/-}$	Pg. FDC 381, Td. ATCC 35404, *T. forsythia* ATCC 43037, F. nucleatum ATCC 49256.	Cytokine array, Western blot	Serum WT	*CASPASE1, MEFV, NLRP1, NLRP3, OR1B1, TICAM2,* IFNG, IFNL3, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 β, IL-17RB, IL-23A, TGF-β1, TNF IL-10, IL-2, IL-4, IL-5	 TGFB1
Velsko et al., 2015 [36]	Mouse	$ApoE^{-/-}$ B6.129P2-Apoetm1Unc/J mice	*F. nucleatum ATCC 49256.*	cytokine array, ELISA, qPCR	Cardiac blood	ADGRE1, CCL2, CCL3, CSF3, CSF2, CX3CL1, CXCL11, CXCL9, FAS, INFG, IGG, IGM, IL-10, IL12B, IL13, IL-1β, IL4, TIMP2, TNFRSF8, XCL1	*THBS4*, CCL11, CXCL13, CXCL5, IL17RB, IL-6, TNF
Xie et al., 2020 [13]	Mouse	$Bmal1^{-/-}ApoE^{-/-}$ jet-legged $ApoE^{-/-}$	*P. gingivalis* strain W38 *P. gingivalis* LPS, *Pg. strain* W38	Flow cytometry qRT-PCR, ELISA, Western blot	Serum Aorta	*IL-1B, IL-6*, IL-1β, IL-6, TNF	 *ARNTL*
Xuan et al., 2017 [38]	Mouse	$ApoE^{-/-}$ (C57BL/6)	*P. gingivalis* FDC381	ELISA, rt-PCR, western blot	Aorta	NOS2, NOX4, OLR1	

(*continued on next page*)

Table 2 (*continued*)

Author Year	Specie	Subject model	Bacteria/ Strain/virulence factor	Methods	Tissue	Upregulated gene or protein	Downregulated gene or protein
Yamaguchi et al., 2015 [33]	Mouse	Apoe$^{-/-}$	*P. gingivalis* KDP136 (gingipain-null mutant) KDP150 (FimA-deficient mutant) *P. gingivalis* ATCC 33277	ELISA rt-PCR	Aorta Peritoneal macrophages	*CASPASE1, IL-18, IL-1β,NLRP3, Pro-caspase1, Pro-IL-18, Pro IL-1β.* IL-18, IL-1β, TNF	
Yang et al., 2016 [42]	Mouse	C57BL/6	*P. gingivalis* ATCC 33277 OVM	Western blot, rt-PCR,	VSMCs	*ALPP, BGLAP, COL21A1, RUNX2*, ALPP, BGLAP, COL21A1, RUNX	*ASMA, TAGLN*

analysis in KEGG "lipids and atherosclerosis" pathway (hsa05417) showed that several genes and proteins modulated by periodontal pathogens and their virulence factors overlapped to molecular events in endothelial cells macrophages and vascular smooth muscle cells (VSMCs), in atherosclerosis.

The impairment of those genes in endothelial cells leads to DNA positive feedback, monocyte attachment and activation, leukocyte transendothelial migration, inflammation and loss of apoptosis mainly mediated by the Toll-like receptor signaling through the nuclear factor κβ (NF-κβ) and NF-kappa-β inhibitor alpha (Iκβα) activation (Fig. 5).

Some of the genes and proteins modulated by periodontal pathogens are involved in the transformation of macrophages in foam cells, the activation of the proCASPASE1 part of the NLRP3 inflammasome and the NOD-like receptor pathway (NLRs). In atherosclerosis, these mechanisms are also activated by low density lipoproteins (LDL) and oxidized low-density lipoproteins (ox-LDL) (Fig. 6). KEGG also showed that in VSMCs genes and proteins involved in apoptosis and plaque instability through modulation of the "p53″ and "TNF-α signaling" pathways were also modulated by periodontal pathogens (Fig. 7). Supplementary Figure 2 shows the integration of the KEGG "lipids and atherosclerosis" pathway for human cells (hsa05417).

We also analysed the KEGG "fluid shear and atherosclerosis" pathway (hsa05418) (Fig. 8) that represents the events associated with atherosclerosis caused by the frictional forces of the blood flow on endothelial cells [27].

This analysis showed the upregulation of modulated genes involved in matrix degeneration, angiogenesis and VSMCs migration, differentiation and proliferation. This added to the leukocyte adhesion and transendothelial migration triggered by the activation of the "NF-κβ signaling" pathway.

The downregulation of BCL2-associated agonist of cell death gene (BCL2) was also visualized. One gene related to anti-atherogenesis mechanisms was also upregulated by *P. gingivalis*, the tissue plasminogen activator (PLAT or t-PA). Similar changes were found in mice, where a gene involved in the metabolisms of lipids was also upregulated (Lectin Like oxLDL receptor 1, LOX1) (Supplementary figure 3).

3.5. Other findings

Studies analyzing the migration of periodontal pathogens to aortic tissue, in animal models, detected genomic DNA of *F. nucleatum, P. gingivalis*, *T. denticola* and *T. forsythia* [28–31]. *Aggregatibacter actinomycetemcomitans, F. nucleatum, P. gingivalis, T. denticola, T. forsythia and Prevotella intermedia* were identified, through the amplification of the 16 S rRNA, in atheromatous plaque from patients with coronary heart disease [13]. *A. actinomycetemcomitans* and *P. intermedia* were not used for gene and protein expression analysis by the included studies.

Some included papers performed histomorphometric analysis of atherosclerotic plaques after exposure to periodontal pathogens in Apoe$^{-/-}$ and TLR2$^{-/-}$TLR4$^{-/-}$ and rabbits [1,10,13,28–38]. Other papers studied different cell events such as chemotaxis, cell proliferation, cell adhesion, foam cell formation, apoptosis, autophagia and calcium influx, in human and animal cells exposed to *P. gingivalis* [13,32,34,38–49]. And the adherence of THP-1 to HAECs when exposed to *E. corrodens* was also studied [50].

4. Discussion

The contributing effect of periodontal pathogens on the molecular mechanisms involved in atherosclerosis has been of special interest [10]. Periodontitis, a chronic inflammatory disease, initiates a systemic inflammatory response that could potentially promote or

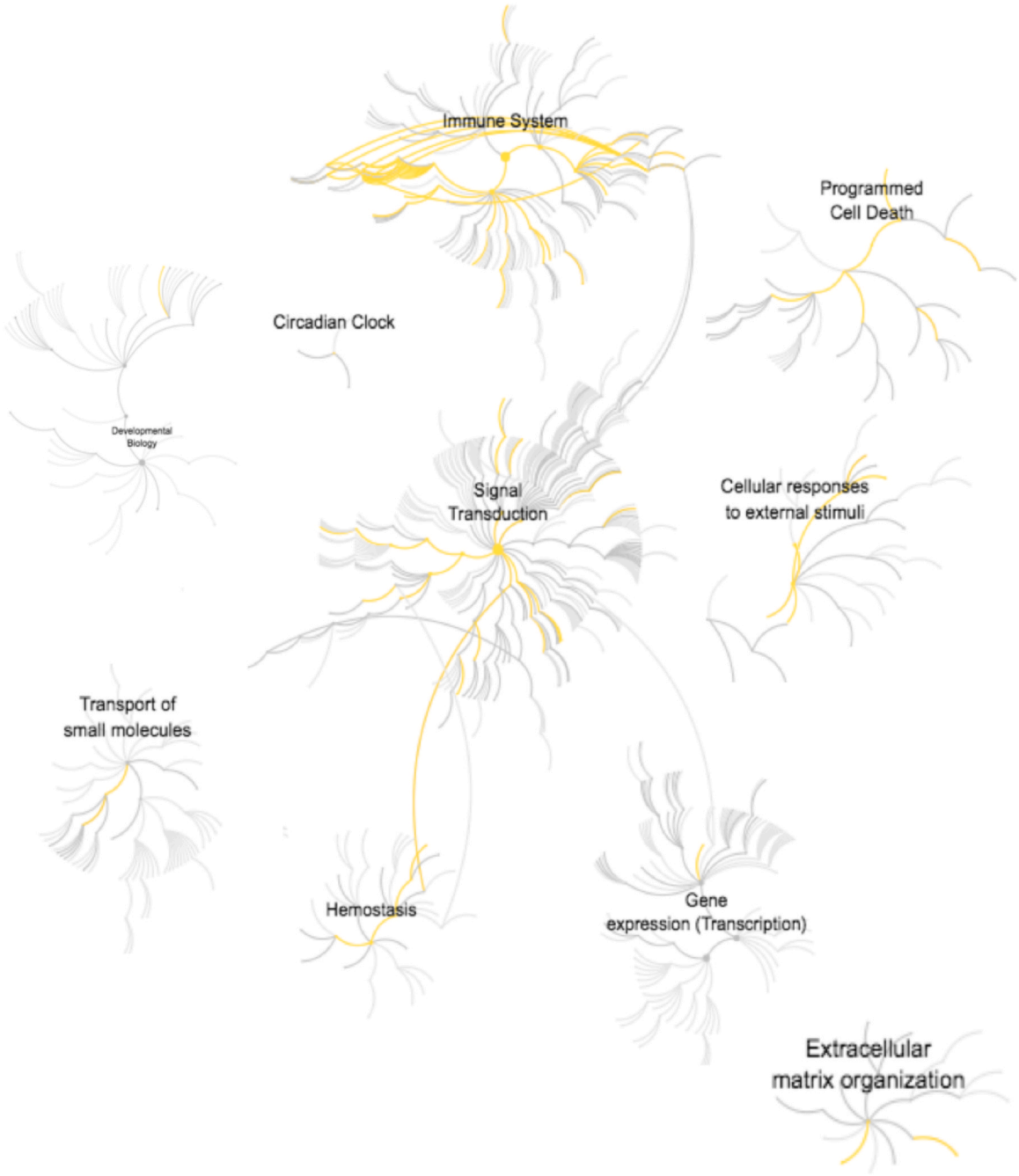


Fig. 3. Overrepresentation analysis humans. Reactome.

exacerbate atherosclerosis, in addition to the transitory bacteremia that occurs in this disease [51]. In this systematic review, the results of previous studies analyzing gene or protein expression in cells, tissues, or animal models of atherosclerosis, were integrated to perform a pathway overrepresentation analysis. This aimed to refine the knowledge about the molecular mechanisms potentially modulated by periodontal pathogens in atherosclerosis.

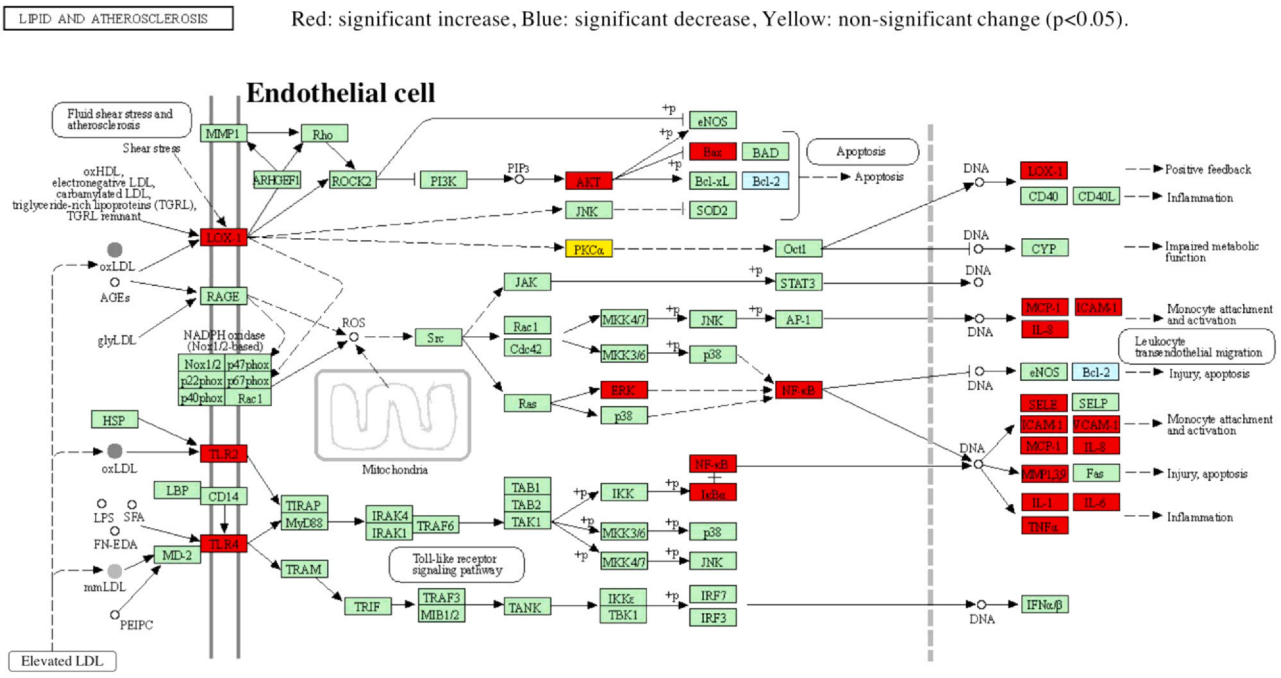
The approach for data analysis in this systematic review is two-fold. First, the results of different studies were integrated by a statistical overrepresentation analysis in Reactome. This analysis revealed superpathways that could be modulated by periodontal pathogens. Second, KEGG allocated the modulated genes to a hierarchical position in atherogenesis-specific pathways (has05417 and has05418) and depicted the effect of the pathogens on these pathways.

The performed analyses, which included only genes and proteins with significant changes in their expression, revealed that inflammation, cell adhesion, necrosis and apoptosis pathways, either in human cells or animal models, were activated by several periodontal pathogens, (Fig. 3, Supplementary Figure 2, 3 and 4).

The included papers analyzed the effect of seven out of 700 bacteria species present in the mouth [52]. These species were: *P. gingivalis*, *T. forsythia*, *F. nucleatum*, *T. denticola*, *E. corrodens, S. anginosus,* and *S. sanguinis*, or their virulence factors, LPS, OMV and

Pathway name	HAECs	HUVECs	AoSMCs	THP1
Diseases associated with the TLR signaling cascade	●	●	●	●
Interleukin-4 and Interleukin-13 signaling	●	●	●	●
MyD88:MAL(TIRAP) cascade initiated on plasma membrane	●	●	●	●
ATF4 activates genes in response to endoplasmic reticulum stress	●	●	●	
Cytokine Signaling in Immune system	●	●		●
Diseases of Immune System	●	●	●	
Immune System	●	●		●
Interleukin-10 signaling	●	●		●
PERK regulates gene expression	●	●	●	
Signaling by Interleukins	●	●		●
Toll Like Receptor 2 (TLR2) Cascade		●	●	●
Toll Like Receptor TLR1:TLR2 Cascade		●	●	●
Toll Like Receptor TLR6:TLR2 Cascade		●	●	●
Cellular responses to stimuli	●	●		
Cellular responses to stress	●	●		
Chemokine receptors bind chemokines	●		●	
CLEC7A/inflammasome pathway	●			●
Innate Immune System		●		●
Interleukin-1 processing	●			●
Intrinsic Pathway for Apoptosis	●	●		
IRAK4 deficiency (TLR2/4)			●	●
MyD88 deficiency (TLR2/4)			●	●
Pyroptosis	●			●
Regulation of TLR by endogenous ligand			●	●
Toll Like Receptor 4 (TLR4) Cascade		●		●
Toll-like Receptor Cascades		●		●
Unfolded Protein Response (UPR)		●	●	
Apoptotic factor-mediated response	●			
ATF6 (ATF6-alpha) activates chaperone genes	●			
ATF6 (ATF6-alpha) activates chaperones		●		
Circadian Clock	●			
Class A/1 (Rhodopsin-like receptors)			●	
CLEC7A (Dectin-1) signaling	●			
Cytochrome c-mediated apoptotic response		●		
Dissolution of Fibrin Clot			●	
GPCR downstream signalling			●	
GPCR ligand binding			●	
Heme signaling	●			
Hemostasis			●	
Interleukin-1 family signaling				●
Interleukin-1 signaling	●			
MECP2 regulates transcription of neuronal ligands			●	
MyD88 dependent cascade initiated on endosome				●
NOD1/2 Signaling Pathway		●		
Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways		●		
Peptide ligand-binding receptors			●	
Programmed Cell Death	●			
Regulated Necrosis	●			
Senescence-Associated Secretory Phenotype (SASP)	●			
Senescence-Associated Secretory Phenotype (SASP)				●
Signal Transduction			●	
Signaling by GPCR			●	
Syndecan interactions			●	
Tie2 Signaling			●	
Toll Like Receptor 7/8 (TLR7/8) Cascade				●
TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation				●

Fig. 4. Top 25 overrepresented pathways in human cells.



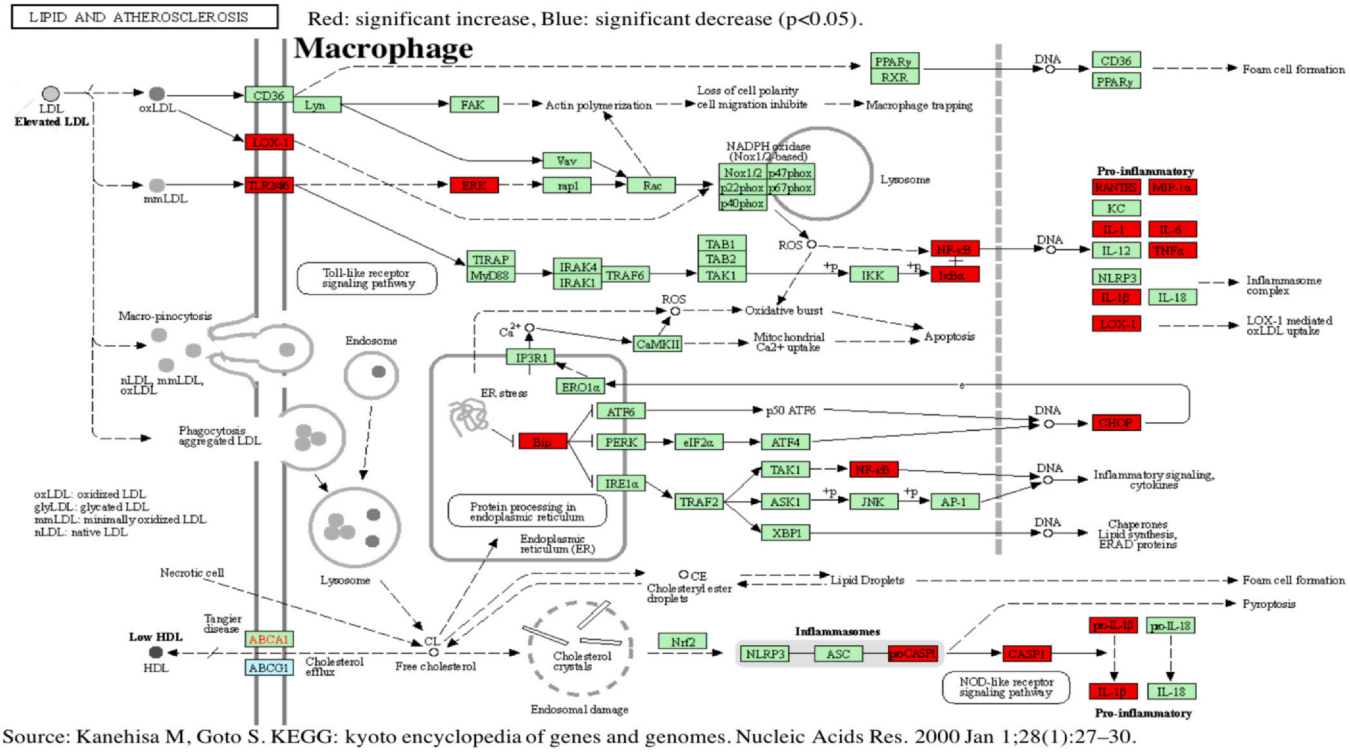
Source: Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27–30.

Fig. 5. KEGG hsa05417 representation of the genes modulated by periodontal pathogens integrating the data for humans in endothelial cells.

gingipains. The effect of these periodontal pathogens in the different stages and molecular mechanisms linked to atherosclerosis will be discussed as follow.

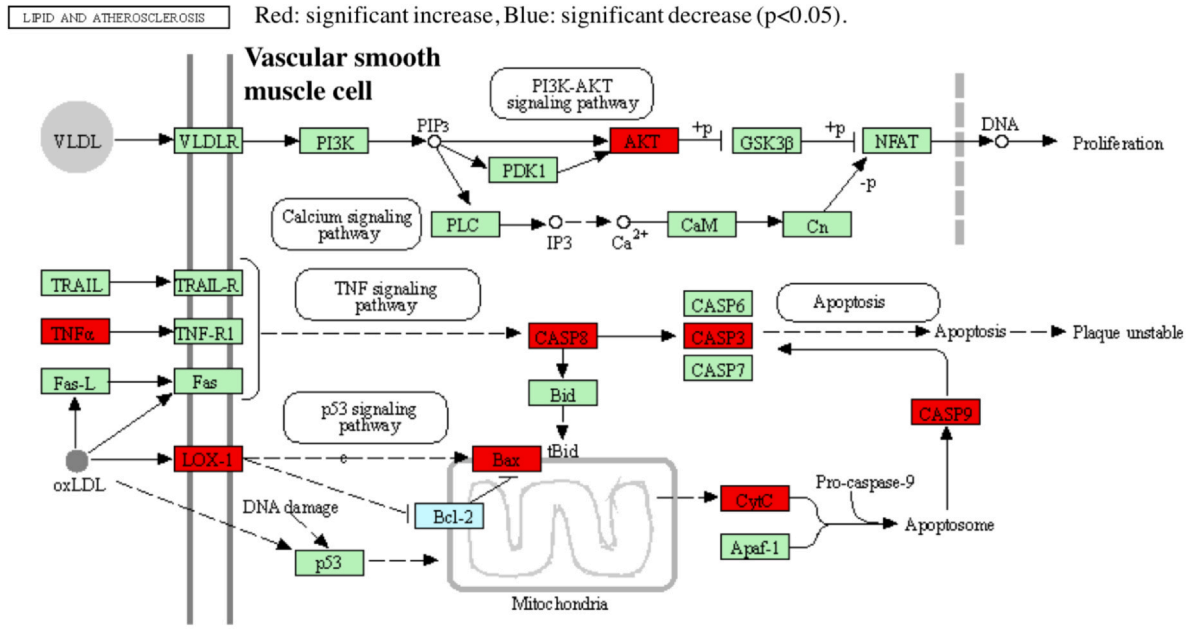
4.1. Endothelial dysfunction and inflammatory cell recruitment

Endothelial dysfunction favors the accumulation of ox-LDL in the intima layer of large arteries [53–55] and has been declared the



Source: Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27–30.

Fig. 6. KEGG hsa05417 representation of the genes modulated by periodontopathogens integrating the data for humans in macrophages.



Source: Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27–30.

Fig. 7. KEGG hsa05417 representation of the genes modulated by periodontopathogens integrating the data for humans in vascular smooth muscle cells.

precursor of atherosclerosis [13,56]. Ox-LDL alters the balance of nitric oxide, activates the innate immune system through the expression of TLR pathways and NLRs, to promote an inflammatory response [57]. By its side, periodontitis has been associated with microvascular dysfunction, altering microvascular responses, decreasing the availability of nitric oxide, increasing the levels of reactive oxygen species (ROS) [58] and it is significantly associated with the expression of biomarkers of endothelial dysfunction and dyslipidemia [59].

The activation of TLR signaling pathways is a pivotal step for the detection of microbial pathogens, generation of innate immune responses and pro-inflammatory cytokines production. TLR2 or TLR4 activates NF-κβ, elevating de concentration of adhesion molecules in endothelial cells, TNF-α expression and endothelial permeability in endothelial dysfunction [3].

In this systematic review, we observed a significant expression of *TLR2* and *TLR4* in HAECs, HUVECs and THP-1 monocytes [45,49,60] (Fig. 4, Supplementary Figure 2) and a significant increase in the chemotactic effect on THP-1 monocytes by *P. gingivalis* [32,39,47]. The studies based on animal models exposed to *P. gingivalis and E. corrodens* reported a significant increase of NF-κβ [1,35,37,61] and those that were exposed to *P. gingivalis, S. sanguinis* and *S. anginosus,* reported a significant increase of TLR2 and TLR4 in peripheral blood and aortic tissue [35,50,61] (Supplementary Figure 3).

It has been stated that endothelial dysfunction is accompanied by the increase of different interleukins, proper of a pro-inflammatory phenotype[62]. TNF-α modulates endothelial cells apoptosis, reduces autophagy and promotes endothelial dysfunction [57]. The included papers in this study confirmed the significant increase of IL-1, IL-6 and TNF-α when HAECs were exposed to *P. gingivalis* [13]. *P. gingivalis* also increased the expression of TNF-α in THP-1 [32,63] and monocytes [64] (Fig. 4 and Fig. 6). In animal models, the serum levels of TNF-α were increased *by P. gingivalis* strain or its LPS [13,28,32,35,61], by *T. forsythia* [10], *T. denticola*, *F. nucleatum* [28] and *S. sanguinis* [37].

In atherosclerosis, ox-LDL also increases the production of chemokines such as the monocyte chemoattractant protein 1 (MCP1), CXCL8, CCL5 (RANTES) and fractalkine (CX3CL1) to attract monocytes, T cells and dendritic cells to the artery wall [43]. In this systematic review, it was found that HUVECs, HAECs and ASMCs exposed to *P. gingivalis* and *E. corrodens* had a significant increase in the expression of these chemokines in absence of ox-LDL ($p < 0.05$) [39,47,50] (Supplementary Table 2).

The pro-inflammatory status in endothelial cells, caused by the exposure to ox-LDL, is accompanied by an increase in the expression of cell adhesion molecules [65]. This facilitates the adhesion and rolling of monocytes, lymphocytes and the deposition of T-cells along the endothelial cell surface [60,66].

The included papers found a significant increase in the expression of the following genes and proteins, E-selectin (SELE) in HUVECs either by *P. gingivalis*, *P. gingivalis* LPS, or *T. forsythia* [13,32,39,47,60], vascular cell adhesion molecule-1 (VCAM-1) in HAECs and HUVECs by *P. gingivalis* [13,32,45]], intercellular adhesion molecule-1 (ICAM-1) in EA.hy926 [46,67], HAECs [13,45,50] and HUVECs [32,39,47] both by *P. gingivalis* and MCP1 in HUVECS *by P. gingivalis* LPS [39], all in the absence of ox-LDL. In animal models, these adhesion molecules were increased by *T. forsythia* [10] and *F. nucleatum* [36]. The increase in the expression of these molecules could be reflected in the THP-1 monocyte adhesion to endothelial cells that was reported by assays performed in-vitro [39,46,47,50].

Data analysis in KEGG showed an important modulation of the "TLR signaling" pathway by periodontal pathogens, also observed in the overrepresentation analysis in Reactome that shows the activation of the "immune system" superpathway (Fig. 3). Added to the upregulation of translocating chain-associated membrane protein 1 (TRAM), TNF receptor-associated factor 6 (TRAF6), NF-κβ and IκBα, plus the modulation of the "adhesion molecules signaling" pathway. This promotes monocyte activation and attachment to endothelial cells, through the expression of SELE, ICAM-1, VCAM-1 and MCP1 and its receptor C-C chemokine receptor type 2 (CCR2) [57] (Supplementary Figure 3 and 4).

4.2. Inflammation in atherosclerosis

The pro-inflammatory environment in atherosclerosis activates macrophages to express pro-inflammatory cytokines and

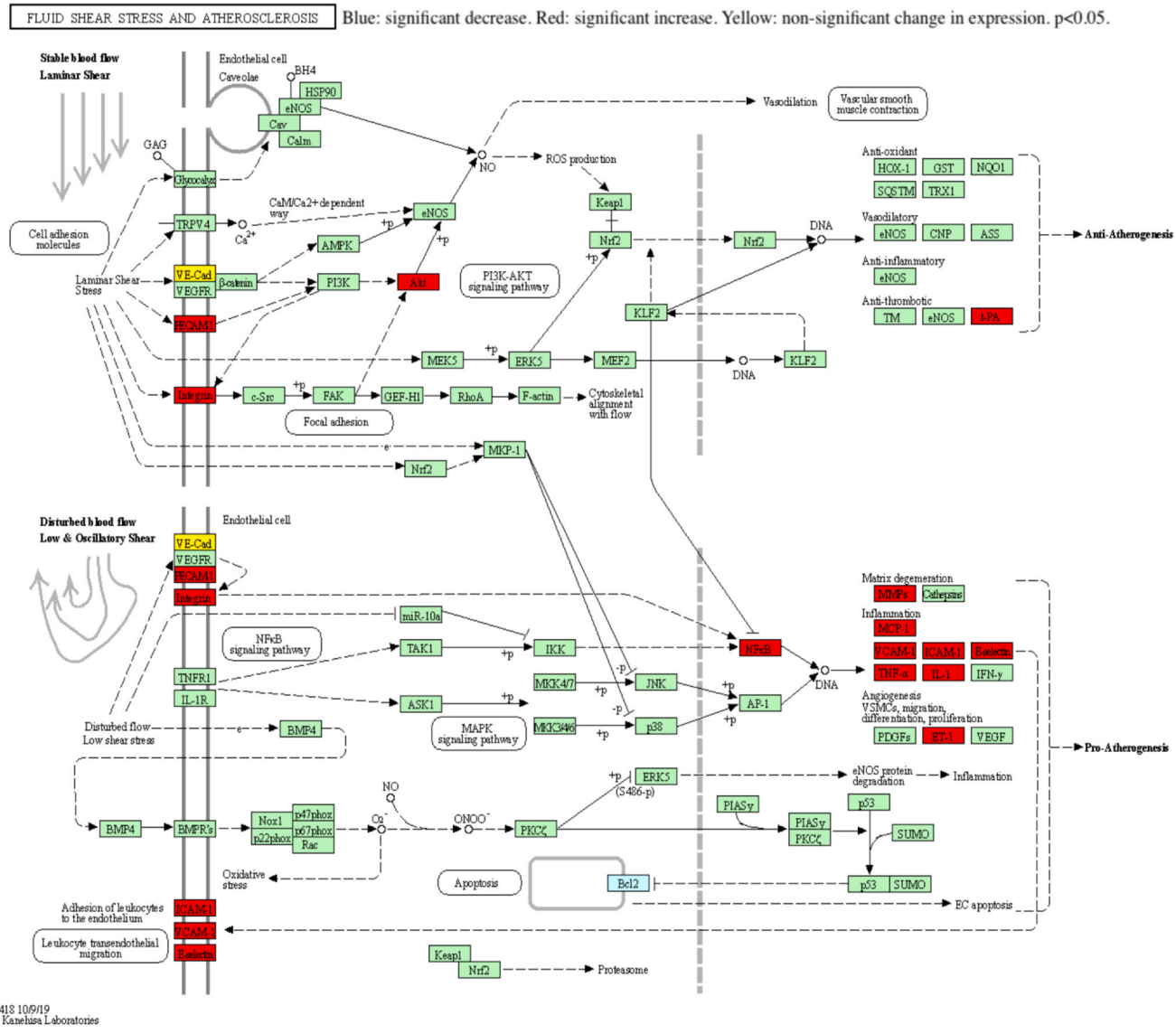


Fig. 8. Humans KEGG. Fluid shear and atherosclerosis pathway (hsa05418).

chemokines. The activated Th1 cells, either by macrophages or by dendritic cells, promote the release of cytokines such as interferon-gamma (INFy), IL12, IL15, IL18 and TNF.

a, deriving in a prolonged inflammatory response [65]. In this systematic review, it was observed that different periodontal pathogens caused a significant increase in the expression of these cytokines, either in THP-1 cells or in animal models [10,13,33–37,61,63,64], as well as the increase of the macrophage migration inhibitory factor (MIF) by *P. gingivalis* in EA.hy926 [46], a proatherogenic cytokine involved in monocyte-endothelial cell adhesion [68].

In atherosclerosis, the affected endothelial cells secrete chemokines for the recruitment and endothelial infiltration of monocytes and T cells into the intima layer, to remove ox-LDL [68,69]. This systematic review found significant changes in the expression of 16 chemokines, either ligands or receptors, when endothelial cells, THP-1, or animal models were exposed to periodontal pathogens [10,29,35–37,39,46,47,49,50,61,64,67,70,71] (Supplementary Table 2).

It is necessary to highlight that the effect of the periodontal pathogens on the expression of these chemokines was variable. *E. corrodens* LPS, decrease the expression of MCP1 and CCL5, in HAECs [50]. *T. forsythia* decreased the expression of CCL5, C-X-C motif chemokine 11 (CXCL11) and C-X-C motif chemokine 13 (CXCL13) in Apoe$^{-/-}$ mouse [10]. *F. nucleatum* decreased the expression of eotaxin (CCL11), C-C motif chemokine ligand 3 like 3 (CCL3), CXCL13 and C-X-C motif chemokine 5 (CXCL5) in Apoe$^{-/-}$ mice [36]. Polymicrobial oral infection with *P. gingivalis*, *T. denticola*, *T. forsythia* and *F. nucleatum* decreased the expression of CXCL11 and C-X-C motif chemokine 2 (CXCL2) in Apoe$^{-/-}$ mice [30]. However, in the same animal model, *S. anginosus* and *P. gingivalis* increased the expression of CCL11[37,71]. *E. corrodens* and a polymicrobial oral infection [30,50] increased CCL5 and *F. nucleatum* increased the expression of CXCL11 [36]. This variability among the bacteria species could be associated with their different virulence factors.

The chemokines reported to be proatherogenic and significantly increased by periodontal pathogens exposure were MCP1 that through CCR2 increases macrophage recruitment into the artery wall. CCL3 regulates cell adherence and infiltration of neutrophils. CCL5 enhances immune cell infiltration in the lesions and monocyte arrest on the endothelium. CXCL8 participates in the development of foam cells, apparently by the inhibition of cholesterol efflux. C-X-C motif chemokine 9 (CXCL9) may facilitate the recruitment and homing of active Th1 cells, by the activation of C-X-C chemokine receptor type 3 (CXCR3) and the C-X-C chemokine receptor type 4

(CXCR4) involved in the neutrophil recruitment that promotes atherosclerotic plaque growth and instability and the chemoattractant leukocyte chemokines CCL11 and CXCL11 that were also increased [68,69,72] (Supplementary Table 2).

Reactome analysis showed that these chemokines were modulated by periodontal pathogens and are part of the overrepresented "chemokine receptors in chemokines" and "IL-10 signaling" pathways (Figs. 3 and 4).

Another relevant mechanism, in the inflammatory process in atherosclerosis, is the activation of the NLRP3 inflammasome in macrophages. This inflammasome can be activated by ROS overproduction [65], by pathogens, or by inflammatory intracellular cholesterol crystals, promoting inflammation and foam cell formation mediated by ox-LDL [1]. *P. gingivalis* activated this inflammasome in Apoe$^{-/-}$ mice and ItgB6$^{-/-}$ mice [28,33].

The activation of NLRP3 leads to the activation of caspase 1 (CASPASE1) that performs the proteolytic cleavage of pro-IL-1β and pro-IL-18, to produce the pro-inflammatory cytokines IL-1 β and IL18, abundant in the atherosclerotic plaque [73].

The pathways analysis performed in KEGG shows that CASPASE1 was expressed by human monocytes and animal models exposed to *P. gingivalis* or *P. gingivalis* LPS [32–34,49,64] (Supplementary Figure 2 and 3). In mice exposed to *F. nucleatum*, *P. gingivalis*, *T. denticola* and *T. forsythia*, the interleukins IL-1β and IL-18 were expressed in the aorta, blood and serum [10,13,28,36,70], an effect that could be associated with the activation of NLRP3 by periodontal pathogens [28,74] (Supplementary figure 3).

4.3. Foam cell formation

One crucial step in the establishment of atherosclerosis is the formation of foam cells from recruited macrophages [43]. LOX1 is activated by ox-LDL and regulates lipoprotein uptake in macrophages [41,43,57,65] leading to foam cell formation. The pathway annotation analysis performed in KEGG integrating the results from HAECs [13,45,50], EA.hy26 [67] and HUVECs [13,32,39,40,45,47,60] exposed to *P. gingivalis* showed a significant increase in *LOX-1* expression, that increases ox-LDL uptake to form foam cells, as well as monocyte migration and adhesion to endothelial human cells [43,45,47] (Supplementary Figure 2).

Additionally, *P. gingivalis* LPS was able to upregulate Acetyl-CoA acetyltransferase (ACAT1) and downregulated ATP-binding cassette (ABC) sub-family G member 1 (ABCG1) in THP-1 [43]. ACAT1 re-esterifies the excess of free cholesterol to be stored in cytoplasmic lipid droplets, bringing the foamy appearance to the recruited macrophages [43] and ABCG1 downregulation reduces the efflux of cholesterol in mice macrophages [75]. Overrepresentation analysis in Reactome shows that ACAT1 is associated with LDL clearance and ABCG1 and participates in HDL remodeling. These events are orchestrated by the "transport of small molecules" superpathway and could be influenced by periodontal pathogens (Fig. 3).

The included studies showed an increase in foam cell formation when animal models, THP-1 human cells and murine resident macrophages from mice were exposed to *P. gingivalis* [63] or *P. gingivalis* LPS [34,41,43]. Foam cell formation, related to *P. gingivalis*, was described as a time-dependent effect and it was greater than the solo exposure to LDL [43].

4.4. Atherosclerotic plaque formation

Fatty streaks are the earliest type of atherosclerotic lesions and are caused by the pro-inflammatory status of the endothelium, inflammatory cell migration and foam cells formation. The persistent recruitment of inflammatory cells, in response to the continuous accumulation of ox-LDL in the intima layer, leads to atherosclerotic plaque formation [65,76].

Section 4.2 described how cell recruitment in atherosclerotic plaque could be modulated by periodontal pathogens and their virulence factors, upregulating chemokines and interleukins. These chemokines are involved in the process of atherosclerotic plaque establishment, from the initiation of a fatty streak lesion to mature plaque formation [69].

Another process involved in the development and growth of atherosclerotic plaque is angiogenesis. It occurs thanks to the migration of smooth muscle cells to the atherosclerotic lesion in response to a hypoxic state in the lesion. Some included papers found that the exposure of ASMCs to *P. gingivalis* had a significant effect on the expression of genes that belong to pathways related to the inflammatory process occurring in atherosclerosis [1,13,32,33,35,37,38,45,61] (Fig. 4).

The exposure of ASMCs to different strains of *P. gingivalis,* like wild-type (W50, 381), gingipain mutant (E8, K1A) and fimbria mutant (DPG-3, KRX-178) demonstrated that gingipains define the ability of *P. gingivalis* to decrease the expression of angiopoietin 1 (Angpt1), an anti-inflammatory regulator and to increase angiopoietin 2 (Angpt2) and its transcription factor ETS1. Angpt2 is a pro-inflammatory and proangiogenic factor, that regulates the angiogenesis process associated with AoSMC migration to the intima layer [44,71].

By its side, cell apoptosis is important in the conformation of the necrotic core of the atherosclerotic plaque. The pathways analysis in KEGG reveals a significant upregulation of genes present in the "TNF-α", "cellular tumor antigen p53 (p53)", "phosphatidylinositol 3′-kinase(PI3K)-Akt (PI3K-AKT)" and "apoptosis" pathways in the ASMCs exposed to periodontal pathogens (Fig. 7). The included papers reported that *P. gingivalis* increased expression of TNF-α and proapoptotic modulators such as phosphorylated AKT (*AKT1),* apoptosis regulator BAX *(BAX),* tumor necrosis factor receptor superfamily member 6 *(FAS),* TNF receptor-associated factor 1*(TRAF1)* and DNA damage-inducible transcript 3 protein *(CHOP)* in HAECs and HUVECs [13,39,40]; together with the downregulation of B-cell lymphoma 2 (BCL2) that regulates cell death and aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL), being the two former, antiapoptotic regulators in HAECs [13].

These changes have a detrimental effect on the endothelial reparative capacity and the stability of the atherosclerotic plaque [13]. Another feature of atherosclerotic plaques is dystrophic calcification that increases cardiovascular mortality [77]. It was reported that *P. gingivalis* LPS and *P. gingivalis* OMV, induced VSMCs calcification, upregulating the expression of RUNX2 an osteogenic transcription factor, alkaline phosphatase (ALPP), integrin-binding sialoprotein (IBSP) and osteopontin (SPP1) in animal models [42,48].

Reactome analysis shows the participation of RUNX2 in the superpathway of "gene expression", regulating osteoblasts differentiation, as well as the connection of SPP1 to this superpathway.

Reactome also reveals how IBSP is part of the extracellular matrix organization superpathway in integrin cell surface interactions (Fig. 3), mechanisms that are likely to be modulated by *P. gingivalis* in atherosclerosis.

The integrative analysis run in KEGG and Reactome showed how the modulated genes participated in pathways that mediate the different stages and mechanisms involved in atherosclerosis.

4.5. Histomorphometric analysis of the atherosclerotic plaque in animal models

Some studies observed the responses in aortic tissue from animals exposed to *P. gingivalis, S. anginosus, S. sanguinis* [37] and *T. forsythia* [10]. Apoe$^{-/-}$ mouse exposed to *S. sanguinis* had a significant increase in atherosclerotic plaque size, similar to *P. gingivalis*. In contrast, *S. anginosus* generate only a slight increase in plaque size [37]. Different studies, using TLR2$^{-/-}$TLR-4$^{-/-}$ mouse, observed that

atherosclerotic plaque size was smaller in comparison with controls when exposed to polymicrobial infections with *P. gingivalis, T. denticola, T forsythia* and *F. nucleatum*, showing that TLR2 and TLR4 activation are important in the atherosclerosis progression mediated by periodontal pathogens [29,31,70]. The exposure of Apoe$^{-/-}$ to *T. forsythia,* caused an increase of the intima layer thickness, with a slight increase of the atherosclerotic plaque [10].

There was a significant increase ($p < 0.01$) in atherosclerotic plaque size in Apoe$^{-/-}$ and Ldlr$^{-/-}$ mice challenged with *P. gingivalis* when compared with the control, either in the aortic tree or in the root [1,13,33,34,38]. In rabbits exposed to *P. gingivalis,* the observed changes included the presence of foam cells and thickening and edema of the intima layer [35,61].

Rabbits exposed to *P. gingivalis* and receiving a high-fat diet presented a decrease of smooth muscle cells, undefined elastic fibers and scattered atherosclerotic plaques, higher than in animals that had a high-fat diet, but that were not exposed to the periodontal pathogen [35,61]. Observations that confirm the modulation of atherosclerosis-related mechanisms and pathways by periodontal pathogens independently of classical risk factors such as dyslipidemia.

5. Conclusion

The integrative analysis of genes and proteins modulated by periodontal pathogens performed in this systematic review facilitates the understanding of the effect of these bacteria on different cells affected in atherosclerosis. Periodontal pathogens can upregulate and enhance the inflammatory mechanisms already present in the atherosclerosis lesion, either migrating to the affected site or increasing the already expressed inflammatory mediators. The bacteria species used in the included studies upregulated mechanisms and pathways related to inflammation, cell adhesion, apoptosis, SMCs calcification, angiogenesis and foam cell formation that are important steps in the formation of atherosclerotic plaques. Based on these results it is possible to state that periodontal pathogens have the potential to be a contributing factor for atherosclerosis even in absence of a high-fat diet or high shear stress. Studies analyzing how the mentioned mechanisms can be targeted for reducing the risk of atherosclerosis in periodontitis patients are recommended.

6. Limitations

It is necessary to state two possible limitations of the performed analysis. First, the pathway overrepresentation analysis was dependent on the analyzed genes by the included studies. For this reason, several genes that belong to these atherosclerosis-related pathways could not be included in our analysis. However, it was possible to detect the effect of the periodontal pathogens on the analyzed pathways. Second, most of the studies were based on monoinfections on cell culture or animal models, a scenario that does not reflect the interactions among the microbiome involved in periodontitis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdsr.2022.12.001.

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