J Periodontol 2013;84(4 Suppl.):S30-S50
doi:10.1902/jop.2013.1340012

Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology

Leticia Reyes[1,†], David Herrera[2,†], Emil Kozarov[3,4], Silvia Roldán[2] and Ann Progulske-Fox[1]

[1]Department of Oral Biology, College of Dentistry and Center for Molecular Microbiology, University of Florida, Gainesville, FL, USA; [2]ETEP (Etiology and Therapy of Periodontal Disease) Research Group, University Complutense, Madrid, Spain; [3]Institute for Cardiovascular Science (CSIC-ICCC), St. Paul Hospital, Barcelona, Spain; [4]Section of Oral and Diagnostic Sciences, Columbia University Medical Center, New York, NY, USA

[†]Contributed equally to this study.

Reyes L, Herrera D, Kozarov E, Roldán S, Progulske-Fox A. Periodontal bacterial invasion and infection: contribution to atherosclerotic pathology.

Abstract

Objective: The objective of this review was to perform a systematic evaluation of the literature reporting current scientific evidence for periodontal bacteria as contributors to atherosclerosis.

Methods: Literature from epidemiological, clinical and experimental studies concerning periodontal bacteria and atherosclerosis were reviewed. Gathered data were categorized into seven "proofs" of evidence that periodontal bacteria: 1) disseminate from the oral cavity and reach systemic vascular tissues; 2) can be found in the affected tissues; 3) live within the affected site; 4) invade affected cell types in vitro; 5) induce atherosclerosis in animal models of disease; 6) non-invasive mutants of periodontal bacteria cause significantly reduced pathology in vitro and in vivo; and 7) periodontal isolates from human atheromas can cause disease in animal models of infection.

Results: Substantial evidence for proofs 1 to 6 was found. However, proof 7 has not yet been fulfilled.

Conclusions: Despite the lack of evidence that periodontal bacteria obtained from human atheromas can cause atherosclerosis in animal models of infection, attainment of proofs 1 to 6 provides support that periodontal pathogens can contribute to atherosclerosis.

Key words: atherosclerosis; autophagy; bacteria; endothelial; infection; invasion; oral; periodontal; review

Accepted for publication 14 November 2012

The proceedings of the workshop were jointly and simultaneously published in the Journal of Clinical Periodontology and Journal of Periodontology.

Introduction

The incidence of atherosclerosis (AS) cannot be fully explained by classical risk factors (Katz et al. 2001). Consequently, the significance of infections as a potential cause of atherosclerosis has gained favour (Ridker 2002, Epstein et al. 2009), which is sustained by an abundance of epidemiological evidence supporting this notion (Ross 1999, Libby et al. 2002). Infectious agents, including periodontal bacteria, have been implicated in the aetiology of various vascular conditions via multiple mechanisms, including direct microbial invasion of endothelial cells. This review will focus on the evidence and significance of cardiovascular host cell invasion by periodontal pathogens in the pathogenesis of atherosclerosis, as well as the different lines of evidence supporting the role of periodontal bacteria in cardiovascular diseases.

Conflict of interest and source of funding statement

The authors declare no conflict of interest. The workshop was funded by an unrestricted educational grant from Colgate-Palmolive to the European Federation of Periodontology and the American Academy of Periodontology.

Overview of cardiovascular disease pathology

Endothelial cells play a predominant role in maintaining optimal cardiovascular function through the production of paracrine factors that modulate vasodilation, inflammation, thrombosis and cellular proliferation in the blood vessel (Vita & Loscalzo 2002). Disruption of endothelial function is one of the earliest indicators of cardiovascular disease,

© 2013 European Federation of Periodontology and American Academy of Periodontology

which can be initiated by a number of factors (Vita & Loscalzo 2002, Pober et al. 2009, Kolattukudy & Niu 2012), including infection (Vita & Loscalzo 2002, Pirro et al. 2008). Chronic periodontal infections may indirectly induce endothelial activation or dysfunction through a state of systemic inflammation as evidenced by elevated plasma acute-phase proteins, Interleukin-6 (IL-6) and fibrinogen (Leivadaros et al. 2005, Buhlin et al. 2009, Higashi et al. 2009, Fedele et al. 2011). Similarly, release of bacterial products into the circulation such as outer membrane vesicles (Bartruff et al. 2005) or gingipains (Fitzpatrick et al. 2009) from *Porphyromonas gingivalis*, or free soluble bacterial components from *Aggregatibacter actinomycetemcomitans* (Oscarsson et al. 2008) can induce pro-atherogenic responses in endothelial cells. More significantly, oral bacteria can also induce endothelial dysfunction through invasion of these cells (Table 1a–c).

Following injury, endothelial cells initiate a series of pro-inflammatory signals such as the release of chemokines, increased expression of cell adhesion molecules that promote attachment and transmigration of leucocytes into the vascular intima (Braunersreuther et al. 2007, Woollard & Geissmann 2010), activation of smooth muscle cells and endothelial cell death programs (Pober et al. 2009). Damaged endothelia also trigger platelet aggregation and initiate thrombus formation at the site of injury, which can result in vessel occlusion (Popovic et al. 2012). Activated leucocytes that have migrated into the subendothelial space continue the inflammatory cycle through production of additional pro-inflammatory cytokines, reactive oxygen species (ROS) and the release of tissue proteinases that degrade the surrounding extracellular matrix. Although monocytes represent a predominant leucocyte within vascular plaques, lymphocytes, neutrophils, mast cells and dendritic cells have also been detected within vascular lesions (Woollard & Geissmann 2010). Smooth muscle cells present within the intima and media layer of the vessel also contribute to vascular pathology by secreting matrix metalloproteinases (Prochnau et al. 2011) and undergoing proliferation (Popovic et al. 2012). Significantly, periodontal bacteria can affect all of these processes either by directly interacting with/invading endothelial cells, smooth muscle cells, leucocytes and platelets, or indirectly by stimulating the release of paracrine factors that modulate the function of these cells.

Bacterial invasion of host cells

Host cell invasion by pathogenic bacteria represents a sophisticated strategy to counter the defences of the human body. Tissue or cellular invasion is a key virulence property for many bacterial species. It provides a "privileged niche" with access to host protein, iron and other nutritional substrates, a shelter from certain immune responses, and a mechanism for persistence in the infected tissue. An intracellular lifestyle also provides the bacterium an opportunity to avoid killing by antimicrobial therapy, as observed with *Chlamydophila pneumoniae* infection (Deniset & Pierce 2010). Certain bacteria have evolved to invade non-professional phagocytic cells. Successful bacterial invasion, defined as survival within the host cell, can be considered to occur in five stages: (1) attachment, (2) entry/internalization, (3) trafficking, (4) persistence and (5) exit. Internalization of a bacterium also results in responses from the cell. Thus, during trafficking, a successful bacterium locates itself, sometimes by modifying cellular compartments or vesicles, and usurps host cell functions to counteract the cell antimicrobial response. Internalized bacteria can remain in a dormant state and/or multiply, resulting in persistent infection. Finally, intracellular bacteria exit either by host cell lysis (Molmeret et al. 2002), or egress via control of a host cell process (Hertzen et al. 2010), or by both (Hybiske & Stephens 2007).

The concept that oral bacteria can invade host cells was controversial when Meyer et al. (1991) provided evidence that *A. actinomycetemcomitans* invaded KB carcinoma cells (Meyer et al. 1991). Since this first report, several studies reporting various aspects of cell invasion by periodontal bacteria have been published (Table 1a–d, 2).

P. gingivalis as a model atherosclerosis organism

Although *P. gingivalis* is not the only periodontopathogenic bacterium implicated in cardiovascular disease, its interactions with cells of the cardiovascular system have been extensively studied and thus provides a model for interactions between a periodontopathogenic species and cardiovascular cells. Invasion of the host cell begins with adherence mediated by a variety of cell-surface adhesins. Since attachment to the target cell must precede entry, *P. gingivalis* adhesion has been extensively studied (Chavakis et al. 2005, Nobbs et al. 2009, Amano 2010, Tribble & Lamont 2010). Its attachment to and invasion of host cells is mediated by multiple adhesins such as the major fimbriae (FimA) for endothelial cells (Deshpande et al. 1998) and macrophages (Hajishengallis et al. 2006). In macrophages, FimA-mediated internalization of *P. gingivalis* involves crosstalk signalling from Toll-like receptor 2 (TLR2) to the β2integrin receptor complex (Harokopakis et al. 2006). Some *fim*A genotypic clones such as type II are found most frequently in vascular tissues followed by types I and IV (Nakano et al. (2008). In addition to fimbriae, other *P. gingivalis* adhesins include gingipains (Amano 2010) and hemagglutinins (Song et al. 2005, Belanger et al. 2012). Kozarov et al. (1998) reported an association between an increased number of repeat domains in hemagglutinin A (HagA) and the invasive potential of *P. gingivalis*. Invasive strains of *P. gingivalis* such as 381, 33277 and W83 contain three or more HagA repeats whereas the non-invasive strain AJW4 has only two repeats. In addition to adhesins, other bacteria in the oral microbiome may be significant since, *Fusobacterium nucleatum* facilitates invasion of endothelial cells by *P. gingivalis* (Saito et al. 2008).

Host cell type, bacterial strain and microbial load directly influence *P. gingivalis* trafficking. *P. gingivalis* invasion of endothelial cells is an active process initiated by the bacterium that requires actin polymerization and a metabolically active cell (Deshpande et al. 1998). *P. gingivalis*

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 1. Bacterial adhesion and invasion: (a) *in vitro P. gingivalis* studies, (b) *in vitro A. actinomycetemcomitans* studies, *in vitro* studies for different species, (d) *in vitro F. nucleatum* studies

Reference	Country	Cells	Bacterial strains	Research methodology
(a)				
Deshpande et al. (1998)	USA	BHEC, BAEC, HUVEC	A7436	Antibiotic protection assay, 1-4 h, metronidazole; SEM, TEM
Dorn et al. (1999)	USA	HCAEC, CASMC, KB	*E. corrodens, P. gingivalis, P. intermedia*	Antibiotic protection assay, 1.5 h, gentamicin and metronidazole; SEM, TEM
Dorn et al. (2000, 2002b)	USA	HUVEC, KB	26 different *P. gingivalis* strains	Antibiotic protection assay, 1.5 h, gentamicin and metronidazole
Rodrigues et al. (2005)	USA	HCAEC	W83	Invasion 2.5 h
Takahashi et al. (2006)	USA	HAEC	381, major and minor fimbriae mutants	Antibiotic protection assay, 1-6 h, metronidazole
Eick et al. (2006)	Germany	KB	ATCC 33277 and seven clinical isolates	Antibiotic protection assay, 1 h, metronidazole
Hajishengallis et al. (2006)	USA	Mouse macrophages	381, non-fimbriated mutant	Cell binding assay, 5-60 min., FACS analysis
Wan & Hajishengallis (2008)	USA	J774A.1 mouse macrophages	381	Cell binding assay, 30 min., FACS; antibiotic protection assay, gentamicin and metronidazole
Hajishengallis et al. (2008)	USA	Human peripheral blood monocytes	33,277	Co-localization, confocal microscopy; antibiotic protection assay, gentamicin and metronidazole
Saito et al. (2008)	Japan	human gingival epithelial (Ca9-22), HAEC	ATCC 33277, W83	antibiotic protection assay, 2 h, gentamicin and metronidazole
Li et al. (2008)	USA	EC, SMC, KB	W83	Antibiotic protection assay, 1.5 h, gentamicin and metronidazole
Zeituni et al. (2009)	USA	Human DC-SIGN(+/-) Raji cell line, human monocyte-derived DCs	381, major (DPG-3)-, minor (MFI)-, or double fimbriae (MFB)-deficient mutants	Cell binding assay, 1–18 h, FACS analysis
Wang et al. (2009)	USA	Mouse macrophages	33,277 (fim-I) and OMZ314 (fim-II)	Cell binding assay, 30 min., FACS
Suwannakul et al. (2010)	UK	Oral squamous cell carcinoma (OSCC) H357; primary gingival epithelial cells	NCTC 11834,W50 and 4 mutants of W50	Antibiotic protection assay, 90 min., metronidazole
Kirschbaum et al. (2010)	Germany	KB (ATCC CCL 17)	ATCC 33277 and M5-1-2	Antibiotic protection assay, 1–18 h, penicillin
Liang et al. (2011)	USA	Mouse macrophages	ATCC 33277 and its KDP128 mutant	Antibiotic protection assay, 30 min., gentamicin and metronidazole
Aruni et al. (2011)	USA	HeLa	W83	Antibiotic protection assay, 45–90 min., metronidazole
Dolgilevich et al. (2011)	USA	HMEC-1, humanmicrovascular endothelial cell; KB (ATCC CCL 17)	W83, AJW2, AJW4, W83 or mutants W83DPG0185, W83DPG0186 and W83D0982	Antibiotic protection assay, 90 min., gentamicin and metronidazole
Zhang et al. (2011)	USA	Primary mouse calvarial osteoblasts	ATCC 33277 or YPF1, fimbriae-deficient mutant	Infection 3 h, confocal microscopy
Carrion et al. (2012)	USA	Human monocyte-derived DCs	381	FACS analysis

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 1. (continued)

Reference	Country	Cells	Bacterial strains	Research methodology
Kinane et al. (2012)	USA	Human gingival epithelial cells	33277; KDP128, RgpA/RgpB/Kgp triple mutant	Antibiotic protection assay, 1–4 h, gentamicin and metronidazole
Moffatt et al. (2012)	USA	Human gingival keratinocytes	ATCC 33277, isogenic DserB, DserB+pserBand serB::FLAG	Infection 10 min., confocal microscopy
Olczak et al. (2012)	Germany	HeLa cells (CCL-2)	A7436, ATCC 33277	Cell invasion assay, 20 h
(b)				
Meyer et al. (1991)	USA	KB, HEp-2, CHO-Kl, RPMI-4788	Various	Antibiotic protection assay, 2 h, gentamycin; OM, TEM
Sreenivasan et al. (1993)	USA	KB, KB-R2A	SUNY 465	Antibiotic protection assay, 2 h, gentamycin; OM, TEM
Meyer et al. (1996)	USA	Mainly KB	SUNY 465, Aa652	Antibiotic protection assay, 2 h, gentamycin; OM, TEM, IF, 24 h persistence
Lepine et al. (1998)	USA, Canada	KB	20 RFLP grouped strains	Antibiotic protection assay, 2 h, gentamycin; IF
Brissette & Fives-Taylor (1999)	USA	KB	12 strains	Antibiotic protection assay, 2 h, gentamycin; IF
Schenkein et al. (2000)	USA	HUVEC	D045D-40	Antibiotic protection assay, 4 h, gentamycin; TEM
Asakawa et al. (2003)	Japan	KB, HGEC	Y4 Serotype b, 16 clinical strains	Antibiotic protection assay, 4 h, gentamycin
Li et al. (2004)	Canada	KB	UT32, various strains	Antibiotic protection assay, 2 h, gentamycin; PCR, cloning, qPCR.
Cao et al. (2004)	USA	KB (HeLa)	VT1169, HK1651, mutants	Antibiotic protection assay, gentamycin; qPCR, IVIAT.
Wu et al. (2006)	USA	KB	Suny 465, derivatives	Antibiotic protection assay, 2 h, gentamycin
Maeda et al. (2010)	Japan	HeLa	R-type, S-type; ATCC 29523	Antibiotic protection assay, 6 h, gentamycin
Arirachakaran et al. (2012)	Thailand	PHGF	ATCC43718 [Y4] serotype b	antibiotic protection assay, 24 h, gentamycin; SEM, TEM
Komatsuzawa et al. (2002)	Japan	No	Y4 Serotype b	Identification of outer membrane proteins
DiRienzo et al. (2002)	USA	KB, HEp-2	Invasive UP54, non-invasive UP6, UP28, CDT- UP57	Kinetics of the response of KB and HEp-2 cells to CDT
(c) *Tannerella forsythia*				
Sabet et al. (2003)	USA	KB	5 strains, including ATCC 43037	Antibiotic protection assay, 5–6 h, gentamycin & metronidazole
Inagaki et al. (2006)	USA	KB	ATCC 43037, BspA-deficient mutant BFM571	Antibiotic protection assay, rh, gentamycin & metronidazole
Sakakibara et al. (2007)	Japan	KB, Ca9-22	ATCC 43037, three deficient mutants	Adherence assay: culture 3 h, immunostaining, CLSM

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 1. (continued)

Reference	Country	Cells	Bacterial strains	Research methodology
Treponema denticola				
Peters et al. (1999)		Endothelial cells	Different *Treponema* spp. Strains	Adherence and penetration assays: ELISA, SEM, TEM
Lux et al. (2001)	USA	HOK-16B	ATCC 35405 and 5 mutantderivatives	Tissue penetration after 8 h
Prevotella intermedia				
Dorn et al. (1998)	USA	KB	Pi17 (fimbriae C), Pi27 (D), ATCC 25611 (A)	Antibiotic protection assay, 1.5 h, gentamycin & metronidazole
Dorn et al. (1999)	USA	KB, HCAEC and CASMC	Pg381 & W50; Pi 17 & 25611; Ec 23834	Antibiotic protection assay, 1.5 h, gentamycin & metronidazole
Iyer et al. (2010)	USA	Mouse NIH 3T3 fibroblast, oral epithelial HN4, HUVEC	Pi17 and	Antibiotic protection assay, 0.5 h, carbenicillin; CLSM
Campylobacter rectus				
Arce et al. (2010)	USA	BeWo, SM9-1	*C. rectus* 314, *C. jejuni*ATCC43457	invasion assay, 5 h; CLSM
Streptococcus mutans				
Abranches et al. (2009)	USA	HCAEC	14 *St. mutans* strains	Antibiotic protection assay, 2 h, gentamycin, penicillin G; TEM, persistence 3, 27 h
(d)				
Winkler et al. (1988)	USA	PF HR-9	Fn2, Pg (ATCC 33277), Streptococci, AaGA3	Adherence assay
Darenfed et al. (1999)	Canada	no	*Fn.nucleatum* ATCC 25586, *vincentii* ATCC 49256, *polymorphum* ATCC 10953	Binding of human plasminogen to *F. nucleatum* subsp. *nucleatum*
Han et al. (2000)	USA	HGEC, KB	5 Fn strains, Pg, Ec, Pi, Tf	Antibiotic protection assay, 3–4 h, gentamycin & metronidazole; TEM
Edwards et al. (2006)	USA	KB, TERT-2	S. cristatus,S. gordonii, S. sanguinis SK36, A. naeslundii, F. nucleatum subsp.polymorphum ATCC 10953	Antibiotic protection assay, 4 h, gentamicin, penicillin G, metronidazole; CLSM
Saito et al. (2008)	Japan	Ca9-22, HAEC	Pg ATCC 33277 & W83, Fn TDC100 & ATCC 25586	Antibiotic protection assay, 2 h, gentamicin, metronidazole
Ikegami et al. (2009)	USA	CHO, TERT	Fn 12230, 2 mutans	Antibiotic protection assay, 3–4 h, gentamicin, metronidazole
Pan et al. (2009)	USA, China	HEp-2	P. *aeruginosa* PAO1, Pg381, Fn ATCC25586, *A. naeslundii* PK606, Aa SUNYAB75, *S. gordonii*Challis CH1	Antibiotic protection assay, 3 h, gentamycin, metronidazole; FISH, cytokines assay
Ji et al. (2010)	Korea	HOK-16B	*S. sanguinis, S. gordonii, V. atypica*, Fn ATCC 25586, Pi ATCC 25611, Pg ATCC 49417, Tf ATCC 43407, Td ATCC 33521	Antibiotic protection assay, 2 h, gentamicin, amoxicillin; TEM, CLSM, qPCR

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 1. (continued)

Reference	Country	Cells	Bacterial strains	Research methodology
Kirschbaum et al. (2010)	Germany	KB	Fn ATCC 25586, Pg ATCC 33277 & M5-1-2, Tf ATCC 43037, Td ATCC 35405. Alone or in combinations.	antibiotic protection assay, 1, 6, 18 h, penicillin; SEM, cytokine and mRNA assays

SEM, scanning electron microscopy; TEM, scanning electron microscopy; CLSM, confocal laser scanning microscopy, OM, optic microscopy; IF, immunofluorescence; RFLP, restriction fragment length polymorphism, Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Td, *T. denticola*; Tf, *T. forsythia*; Pm, *P. micra*; Pi, *P. intermedia*; Fn, *F. nucleatum*; Ec, *E. corrodens*; Cr, *C. rectus*, Pi, *P. intermedia*; Ec, *E. corrodens*. Cell lines: BAEC, bovine aortic endothelial cells; BeWo, human trophoblast cell line derived from a human choriocarcinoma; BHEC, bovine heart endothelial cells; Ca9-22, epithelial cell-like gingival carcinoma cell line; CASMC, coronary artery smooth muscle cell; CHO-Kl, Chinese hamster ovary; EC, endothelial cells; HAEC, human aorta endothelial cells; HCAEC, human coronary artery endothelial cells; HeLa, cervical cancer cells; HEp-2, human larynx epithelial cells, originally derived from an epidermoid carcinoma from the larynx, now known to contain contaminant HeLa cells; HGEC, human gingival epithelial cells; HN4, oral epithelial cells; HOK-16B, human gingival keratinocyte cell line; HUVEC, monolayers of human vascular endothelial cells; KB, originally thought to be derived from an epidermal carcinoma of the mouth, have now been shown to be derived from HeLa cell cultures as a contaminant; KB-R2A, a cell line defective for endosomal acidification; NIH 3T3, mouse fibroblast cells; PF HR-9, induced differentiation of an embryonal carcinoma; PHGF, primary human gingival fibroblasts; RPMI-4788, human intestinal epithelial cells; SMC, smooth muscle cells; SM9-1, mouse trophoblast cell line, derived from a gestational day 9 Swiss–Webster mouse placenta; TERT-2; oral keratinocyte cells.

Table 2. Bacterial adhesion and invasion: *in vivo* studies

Reference	Country	Samples	Patients	Target strains/species	Invasion methods
Saglie et al. (1986)	USA, Chile	Gingival biopsies	6 SevP, 2 LJP, 2 healthy	Aa, Pg, *Capnocytophaga gingivalis*	OM, TEM, immunoperoxidase
Christersson et al. (1987a)	USA	35 (+4 control) biopsies	12 LJP, 2 healthy, 1 ChP, 1 monkey	Aa-three serotypes	IF (rabbit antisera), TEM
Christersson et al. (1987b)	USA	11 (+3 control, Aa sites) biopsies	6 LPJ	Aa	Culture after serial washing
Noiri et al. (1997)	Japan	12 teeth	7 SevChP	Pg, Cr, *A. viscosus*	Immunohistochemical staining
Rudney et al. (2001)	USA	Cheek samples	24p, 13 m, 11f, 1edentulous	Aa, Pg	FISH & CLSM; multiplex PCR
Rudney et al. (2005)	USA	Cheek samples	38p, 20 m, 18f	Aa, Pg, Tf	FISH & CLSM; qPCR
Leung et al. (2006)	USA	Supra, sub, cheek samples	27p, before and 1 m after ortho	Aa, Tf, Streptococci	FISH & CLSM; qPCR
Colombo et al. (2006)	Brasil, USA	6 samples (3 pocket, 3 sulcus) per patient	49 ChP	33 species	Checkerboard
Colombo et al. (2007)	Brasil, USA	58 samples (pockets, sulcus, buccal mucosa)	22p, 14 Periodontitis, 8 Healthy	Aa, Pg, Tf, Td	FISH & CLSM
Johnson et al. (2008)	USA	buccal mucosa and subgingival samples	18 AgP (treatment SRP, AmoxiMet, CHX)	Aa, Pg, Tf, Td, Pi	FISH & CLSM; qPCR (baseline, 3 m, 6 m)

OM, optic microscopy; SEM, scanning electron microscopy; TEM, scanning electron microscopy; IF, immunofluorescence; FISH, fluorescence in situ hybridization; CLSM, confocal laser scanning microscopy, Sev, severe; Ch, chronic; P, periodontitis; LPJ, localized juvenile periodontitis; m, male; f, female; SRP, scaling and root planing; AmoxiMet, amoxicillin plus metronidazole; CHX, chlorhexidine, Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Td, *T. denticola*; Tf, *T. forsythia*; Pm, *P. micra*; Pi, *P. intermedia*; Cr, *C. rectus*.

internalizes via lipid rafts in human aortic endothelial cells (Yamatake et al. 2007) and traffics via the autophagic pathway in cardiovascular endothelial and smooth muscle cells (Dorn et al. 2001); in contrast it utilizes the endocytic pathway during invasion of oral epithelial cells (Takeuchi et al. 2011) (see Fig. 1). However, only certain strains of *P. gingivalis,* such as 381 and W83, usurp the autophagic pathway during invasion of endothelial cells (Dorn et al. 2001). Invasion via the autophagic pathway is observed at a low multiplicity of infection (100 MOI), which is more consistent with bacterial numbers likely to meet endothelial cells because of bacteremic events. At a higher MOI of 1000, the majority of *P. gingivalis* organisms travel through endosomes instead of autophagosomes (Yamatake et al. 2007) which suggests that bacterial dose plays a role in trafficking of *P. gingivalis*. In epithelial cells, *P. gingivalis* exits through the endocytic recycling pathway (Takeuchi et al. 2011). It remains unknown if *P. gingivalis* exit from cardiovascular cells (Li et al. 2008) is through the same pathway. Regardless of the pathways employed by *P. gingivalis,* these strategies are likely to be critical for microbial dissemination and evasion of host cell clearance mechanisms and pathological changes within the cardiovascular system.

© 2013 European Federation of Periodontology and American Academy of Periodontology

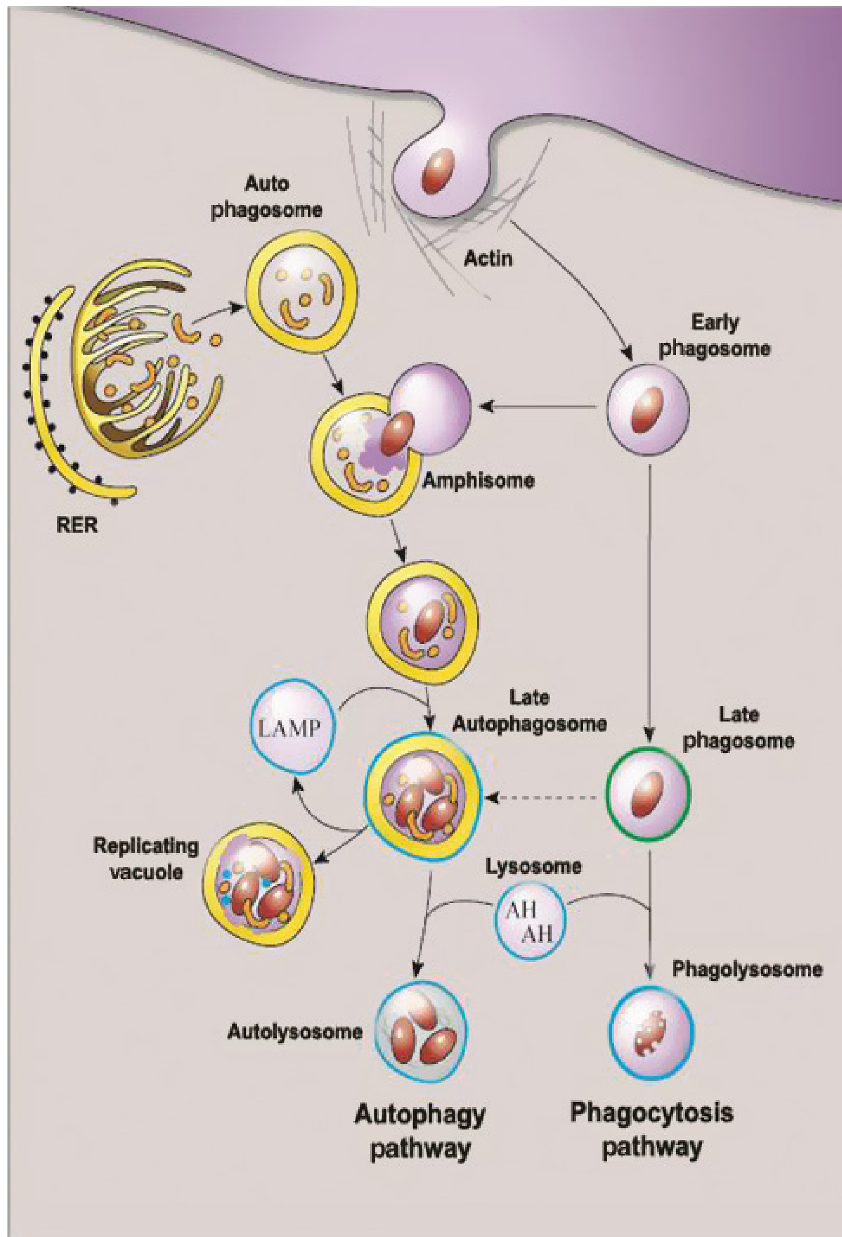

Fig 1. Model of *P. gingivalis* invasion of endothelial cells. Schematic of *P. gingivalis* interactions with the autophagic pathway in human cardiovascular aortic endothelial cells.

As stated earlier, infection with invasive *P. gingivalis* induces monocyte migration and significantly enhances production of the pro-inflammatory cytokines (Pollreisz et al. 2010). *P. gingivalis* also induces pro-coagulant effects in human aortic endothelial cells (Roth et al. 2006) and causes apoptosis of and increases mononuclear cell adhesion to endothelial cells (Roth et al. 2007a,b). *P. gingivalis* transmission occurs in vitro between primary vascular cell types, suggesting that *P. gingivalis* can traverse from one cell to another in vivo, such as from endothelial cells to smooth muscle cells as the atherosclerotic process progresses (Li et al. 2008). Critical to infectious transmission, *P. gingivalis* survival within human vascular cell types (Li et al. 2008), as well as uncultivable *P. gingivalis* could reactivate after their internalization by phagocytes (Rafferty et al. 2011).

Other periodontal pathogens in atherosclerosis

Although invasion of the endothelium and other vascular cells by *P. gingivalis* can certainly account for much of the atherosclerotic pathology, there is data indicating that atherosclerosis is a multi-species infection. Evidence for the detection of multiple periodontal species in individual atheromas began with the first reports of the detection of oral bacterial genomes within atheromas (Table 3) (Haraszthy et al. 2000a,b). The fact that multiple oral bacterial species are associated with atherosclerosis is neither unreasonable nor unexpected, given the species complexity in dental plaque. The adherence/invasion properties of these and other periodontal pathogens follow (see Table 1b–d for in vitro studies and Table 2 for in vivo studies).

A. actinomycetemcomitans

A. actinomycetemcomitans invasion efficiency of epithelial cells is dependent on bacterial strain or microbial colony morphology, and microbial entry into host cells can occur through several receptors (Meyer et al. 1991, 1996, Meyer & Fives-Taylor 1997). For example, *A. actinomycetemcomitans* may be gaining entry into host cells through engagement with transferrin and integrin receptors (Meyer & Fives-Taylor 1997). Moreover, *A. actinomycetemcomitans* entry into host cells is strain variable in that some strains enter host cells through an actin-dependent mechanism, while other strains employ an actin-independent entry into host cells (Brissette & Fives-Taylor 1999, Fives-Taylor et al. 1999). During the past decade, several research groups have added to our knowledge of *A. actinomycetemcomitans* invasion (see Table 1b). Among these, it was demonstrated that *A. actinomycetemcomitans* invasion of human vascular endothelial cells involves an interaction between bacterial phosphorylcholine (PC) and the platelet-activating factor (PAF) receptor of the host cell (Schenkein et al. 2000).

Prevotella intermedia

P. intermedia (strain 17) can invade coronary artery cells (both endothe-

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 3. List of "proofs" that has to be fulfilled to demonstrate that periodontal bacteria are a contributing factor to atherosclerosis

Proof	Description
1	Periodontal bacteria can reach systemic vascular tissues.
2	Periodontal bacteria can be found in the affected tissues.
3	Evidence of live periodontal bacteria at the affected site.
4	In vitro evidence of invasion of affected cell types.
5	Demonstration that periodontal bacteria can promote atherosclerosis in animal models of disease.
6	In vitro and in vivo evidence that non-invasive mutants cause significantly reduced pathology (animal model).
7	Fulfil modified Koch's postulate to demonstrate that a human atheroma isolate causes disease in animal models.

lial and smooth muscle cells) in vitro (Table 1c). The type *C. fimbriae* and the invasin protein, AdpC (belonging to the leucine-rich repeat protein family) appear to mediate the invasion process.

Tannerella forsythia

Multiple *T. forsythia* strains attach to and invade epithelial cells in vitro, mediated by the surface layer composed of proteinaceous arrays (Sabet et al. 2003, Sakakibara et al. 2007) (Table 1c). Similar to *P. intermedia*, epithelial cell attachment and invasion are also dependent on a cell-surface associated leucine-rich repeat protein, BspA. Interestingly, *P. gingivalis* or its outer membrane vesicles enhance *T. forsythia* attachment and invasion (Inagaki et al. 2006). At present, there is minimal information about *T. forsythia* interactions with cardiovascular cells.

F. nucleatum

Many strains of *F. nucleatum* are invasive in vitro (Table 1d). The *F. nucleatum* adhesin, FadA, appears to be required for both attachment and invasion (Ikegami et al. 2009). *F. nucleatum* may also transport non-invasive species (e.g. streptococci) into host cells via a combination of co-aggregation and invasion mechanisms and also facilitate *P. gingivalis* invasion of human gingival epithelial and endothelial cells (Saito et al. 2008), but evidence of *F. nucleatum* co-aggregation among different combinations of species including *P. gingivalis, T. denticola* or *T. forsythia* is equivocal (Kirschbaum et al. 2010).

Other oral bacterial species

There is limited in vitro (Table 1a–d) or in vivo (Table 2) evidence that other bacterial species, such as *Treponema denticola* and *Campylobacter rectus,* have been detected in atheroma specimens or demonstrated to invade host cells.

Emerging mechanisms of atherosclerosis: autophagy as an endothelial stress response and its perturbation by *P. gingivalis*

Autophagy represents a reparative cellular response that degrades senescent cellular organelles, damaged intracellular proteins, and invading intracellular bacteria (Dorn et al. 2002a, Deretic 2011).

There is emerging evidence that the autophagic response may influence cardiovascular disease outcomes during atherosclerosis by affecting plaque stability (Martinet & De Meyer 2008, Schrijvers et al. 2011). For instance, increased autophagic activity measured by LC3 activation or expression have been detected within atheromatous plaques from humans and murine models of sterile atherosclerosis (Martinet & De Meyer 2008, Liao et al. 2012). Further, ApoE-null mice fed high fat diets exhibit impaired autophagy within the atheromatous plaque as disease progresses (Liao et al. 2012), and impaired autophagy has been linked to increased inflammation, which has pro-atherogenic effects (Razani et al. 2012).

In sterile atherosclerosis, autophagy is considered a protective mechanism that prevents apoptosis in cells that are undergoing oxidative stress (Scherz-Shouval & Elazar 2007, Xie et al. 2011) or endoplasmic reticulum stress (Bernales et al. 2006, Zhang et al. 2010a), which is a common feature of atherosclerotic disease. However, in vitro studies have demonstrated that over-activation of autophagy or dysregulation of the autophagic pathway in cardiovascular cells can also be detrimental (Larocca et al. 2012) by promoting cell death referred to as "necroptosis" (Khan et al. 2012, Kolattukudy & Niu 2012), which is potentially pro-atherogenic due to its pro-inflammatory effect (Kolattukudy & Niu 2012).

As an innate immune clearance mechanism, autophagy can be activated in response to signalling via toll-like receptors (TLRs), Nod-like receptors (NLRs) and/or the sequestasome (Shin et al. 2010, Anand et al. 2011, Deretic 2011). Some intracellular pathogens including *P. gingivalis* have developed strategies to subvert this line of defence (Deretic & Levine 2009). *P. gingivalis* strain 381 usurps the autophagic pathway during invasion of human coronary artery endothelial cells and smooth muscle cells in vitro, as shown in Fig. 1 (Dorn et al. 2001). Specifically, bacteria delay fusion of the autophagosome with lysosomes thus, interfering with autophagic flux. This process may impair the ability of the host cell to use the autophagic response as a means of rescuing the cell from oxidative or endoplasmic reticulum stress. Rodrigues et al. (2012) have recently demonstrated that not all *P. gingivalis* strains manipulate this pathway during host cell invasion; W83 perturbs the autophagic pathway of endothelial cells in a similar fashion as 381, whereas strains A7436 and 33,277 do not.

Evaluation of the evidence

In a clinical setting, it is extremely difficult to establish the causative factor of atherosclerosis for several reasons. First, the initiating factor is likely to be missed since the early phase of endothelial injury is usually asymptomatic (Vita & Loscalzo 2002). Second, the atherosclerotic lesion is a common inflammatory response to multiple factors (Keizer 2012, Raman et al. 2013), and some or all of those factors may be associated with the lesion at the time of discovery. Third, interventional studies that evaluated the impact of periodontal treatment, with or without antimicrobial therapy, on systemic inflammation or endothelial dysfunction, have shown mixed results

© 2013 European Federation of Periodontology and American Academy of Periodontology

including no change, transient worsening of signs immediately after treatment, or improvement in signs that did not necessarily persist over time (extensively reviewed by Kebschull et al. 2010). Despite these limitations, it is possible to, at least, demonstrate biological plausibility that invasion of cardiovascular tissues by periodontal bacteria have the potential to promote atherosclerosis through fulfilment of several proofs, discussed elsewhere in the article (Table 3).

Proof that periodontal bacteria can reach systemic vascular tissues

There is no doubt that oral bacterial species can enter the circulation and cause bacteremia (Table 4a–d), which has been documented by several groups. There are potentially multiple mechanisms for systemic dissemination of periodontal bacteria:

- As the gingival pocket is separated from gingival micro-capillaries by a few cells, it is thought that periodontal species that invade oral cells can cross this layer and enter the circulation via a transcellular mechanism (Takeuchi et al. 2011).
- More often, however, periodontal bacteria likely enter the circulation following physical perturbations of the gingiva. Among these perturbations, some are related to dental procedures such as tooth polishing, scaling, tooth extraction, surgical extraction of third molar and periodontal probing (see Table 4a–d). Other perturbations are daily life activities, including tooth brushing, flossing, chewing or biting an apple, as listed in Table 4a–d. The frequency of bacteremia showed a wide variability, depending on the study design, the microbiological methodology, the stimulus and the periodontal health status of the patients. In some studies, an association with plaque and/or gingival indices has been reported (Silver et al. 1977, Forner et al. 2006, Lockhart et al. 2009). A recent systematic review with meta-analysis on the prevalence of bacteremia following tooth brushing, evaluated the pooled odds ratios of the influence of plaque and gingival indices at 2.61 and 2.77 respectively (Tomas et al. 2012). In addition, as shown in Table 4a–d, multiple studies have detected different bacteremic periodontal pathogens, especially after subgingival debridement procedures in periodontitis patients. However, these results should be interpreted with caution due to variability among these studies, which may be due to limitations of the methodology that was used that may affect the sensitivity and detection thresholds (culture-based methods). In addition, well-validated PCR-based methods were not frequently used.
- Another proposed but unproven mechanism of bacteremia is that periodontal bacteria enter the circulation and disseminate to distant sites via survival in immune cells (the Trojan horse approach) (Carrion et al. 2012). In such cases, phagocytosis is beneficial to the bacterial pathogen but detrimental to the host. In this scenario, the internalized pathogen evades microbial killing within the leucocyte and is able to escape from the phagocyte after the cell reaches another site within the body (Zeituni et al. 2009). For example, *P. gingivalis* gains entry into dendritic cells and disrupts their phagocytic function as evidenced by retention of an immature phenotype characterized by a low production of inflammatory mediators (Zeituni et al. 2009, 2010). Regardless of mechanism, there is no doubt that oral pathogens frequently enter the circulation.

Proof that periodontal bacteria can be found in the affected tissues

Since bacteremias of oral origin are frequent, the association of periodontal pathogens with atherosclerotic pathology has been the focus of multiple studies. A variety of oral bacterial species have been identified in atheromatous tissues at the DNA, RNA or antigen level. Haraszthy et al. (2000a) were the first to report the detection of the genomic DNA of *A. actinomycetemcomitans, P. gingivalis* and other species using bacterial 16S rRNA – specific PCR analysis. These PCR detection studies were further extended and confirmed by others (Tables 5, 6). Interestingly, most of these reports found evidence of multiple species in individual atheromas. Thus, there is sufficient data to conclude that multiple pathogenic species reach the affected site. However, the impact of these results are somewhat hampered by several factors. For example, the presence of bacterial components (DNA, RNA and antigens) does not distinguish between live and dead bacteria within the tissue. Further, some species of bacteria,, such as *Veillonella sp*, which have been detected within atheromas (Koren et al. 2011), are not cultivable (Chalmers et al. 2008). Finally, not all studies have detected microbial products within the atheroma, and this lack of consistency may be due to differences in methodology (Figuero et al. 2011) or underlying aetiology of the atheroma.

Evidence of live periodontal bacteria at the affected site

For several years, multiple groups attempted to culture periodonto-pathogenic organisms from atheromas to no avail. Finally, Kozarov et al. (2005) reported evidence of live *P. gingivalis* and *A. actinomycetemcomitans* in atheromatous tissue. This was accomplished by incubating homogenized atheromatous tissue in culture with primary human coronary artery endothelial cells (HCAECs). After several days of incubation, the group used fluorescently labelled antibodies and deconvolution microscopy to visualize intact bacteria within the endothelial cells. Because both species must be alive in order to invade, the presence of the bacteria within the non-phagocytic cells provided strong evidence of the existence of live bacteria in the atheromas. In addition, Rafferty et al. (2011) cultured atheroma samples with macrophages as an intermediate step and was able to isolate *P. gingivalis* on culture plates. These studies require fresh atheromas (angioplasty and stenting have become the standard of care) and the complexity of the protocol appears to have precluded confirmatory reports to date. Thus, although periodontal pathogens cannot routinely be cultured from atheromas directly onto plates, there

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 4. (a) Clinical studies evaluating bacteremia of oral origin: frequency and main microbiological findings, (b) Randomized clinical trials evaluating bacteremia of oral origin: frequency and main microbiological findings, (c) Cohort studies evaluating bacteremia of oral origin: frequency and main microbiological findings, (d) Validation studies evaluating bacteremia of oral origin: frequency and main microbiological findings

Reference	Main technique	n	Patients	Intervention	Baseline	Post-intervention	1st draw	2nd draw	3rd draw	Main species	Periopathogens (n patients)
(a)											
Silver et al. (1977)	Culture	96	Gingivitis	Powered brushing	2.10%	During	42.70%			NR	*B. melaninogenicus*(7), Pepto(3)
Silver et al. (1979)	Culture	36	Healthy	Powered brushing	0.00%	During	8.30%			NR	No
Heimdahl et al. (1990)	Lysis filtration	20	Adults	Extraction	0.00%	During, 10 min.	95.00%	40.00%		*St. viridans*, anaerobes	No
		20	Adults	Third-molar surgery	0.00%		55.00%	40.00%		*St. viridans*, anaerobes	Pm(2)
		20	Periodontitis	Scaling	0.00%		65.00%	30.00%		*St. viridans*, anaerobes	Fn(1)
		20	Adults	Endodontic	0.00%		15.00%	5.00%		*St. viridans*, anaerobes	No
		20	Adults	Tonsillectomy	0.00%		55.00%	0.00%		*St. viridans*, anaerobes	Pg(1), Fn(1)
Messini et al. (1999)	Culture	18	Disable	Various procedures	Not done	Immed, 5 min., 30 min.	83.30%			*Gemella*, Streptococci	Pg(4). Pm(1)
Rajasuo et al. (2004)	Culture	16	Young adults	Third-molar surgery	NR	10, 15, 30 min.	44.00%	25.00%	13.00%	Streptococci	Prevot., *Eubacterium* sp., Pepto
Kinane et al. (2005)	BACTEC	30	Periodontitis	Probing	6.70%	30 s–1 min.	20.00%			NR	Pi(1)
			Periodontitis	Manual brushing	NR	<3 min.	3.30%			NR	No
			Periodontitis	Scaling	No	Immed	13.30%			NR	No
Tomas et al. (2007a)	BACTEC	53	Disable	Extraction (under GA)	NR	30 s, 15 min., 1 h	96.20%	64.20%	20.00%	Streptococci (63.8%)	Fn(1), Pm(2)
Lafaurie et al. (2007)	Culture	42	Periodontitis	Scaling	NR	Immed, 15, 30 min.	80.90%		19.00%	NR	Pg(12), Pm(7), Ec(4), Tf(3), Pi(3), Fuso(5)
Brennan et al. (2007)	NR	100	Children	Various procedures	NR	Eight draws	NR	NR	NR	NR	No
Tomas et al. (2008)	BACTEC	100	Adults	Third-molar surgery (GA)	NR	30sec, 15 min.	62.00%	67.00%		*St. viridans*	None
Lucas et al. (2008)	Lysis filtration	32	Children & adolescents	Manual brushing	NR	30 s after	22.00%	19.00%		Variety, no anaerobes	No
		35		Powered brushing OralB	NR		26.00%	34.00%		Variety, no anaerobes	No
		33		Powered brushing Sonicare	NR		27.00%	33.00%		Variety, no anaerobes	No
		41		Tooth polishing	NR		15.00%	37.00%		Variety, no anaerobes	No
Perez-Chaparro et al. (2008)	Culture	16	Periodontitis	Scaling	NR	Immed, 15, 30 min.	NR	NR	NR	Focused on Pg	7 of 16, Pg-positive

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 4. (continued)

Reference	Main technique	n	Patients	Intervention	Baseline	Post-intervention	1st draw	2nd draw	3rd draw	Main species	Periopathogens (n patients)
(b)											
Diz Dios et al. (2006)	BACTEC	53	Disable	Extractions (GA) & placebo	NR	30 s, 15 min., 1 h	96.00%	64.00%	20.00%	Streptococci	Fn(1), Pm(2)
		56		Extractions (GA) &amoxi	NR		46.00%	11.00%	4.00%	Streptococci	Prevot(4), Pepto(4), Ec(2)
		54		Extractions (GA) &clinda	NR		85.00%	70.00%	22.00%	Streptococci	Fuso(2), Pepto(2)
		58		Extractions (GA) &moxiflo	NR		57.00%	24.00%	7.00%	Streptococci	Fuso(2), Prevot(4), Pepto(8), Ec(2)
Cherry et al. (2007)	Lyso-centrifugation	30	Gingivitis	Scaling & rinse povidone	3.30%	30 sec, 2 min.	3.30%	6.70%		*St. viridans*	No
		30		Scaling & rinse saline	10.00%		13.30%	30.00%		*St. viridans*	Pi(4), Pg(1)
Tomas et al. (2007b)	BACTEC	53	Disable	Extraction (GA) control	9.00%	30 s, 15 min., 1 h	96.00%	64.00%	20.00%	Streptococci (63.8%)	Fn(1), Pm(2)
		53		Extraction (GA) preCHX	7.00%		79.00%	30.00%	2.00%	Streptococci (68%)	Pm(1)
Bahrani-Mougeot et al. (2008)	BACTEC, 16s RNA sequencing	98	Adults	Manual brushing 2 min.	NR	During, immed, 20, 40, 60 min.	NR	NR	NR	Streptococci	Pm(3), Pi(1), Dn(1)
		96		Extraction &amoxi	NR		NR	NR	NR	Streptococci	Pm(2), Pi(9), Dn(1)
		96		Extraction & placebo	NR		NR	NR	NR	Streptococci	Pm(16), Pi(3), Dn(6)
Lockhart et al. (2008)	BACTEC, 16s RNA sequencing	98	Adults	Manual brushing 2 min.	3 cases	During, immed, 20, 40, 60 min.	Cumulative 32%	60 min., 9%	*Streptococcus* (49%), *Prevotella* (9%), *Actinomyces* (5%), *Fusobacterium* (5%).	*Prevotella* (9%), *Fusobacterium* (5%).	
		96		Extraction &amoxi			Cumulative 56%	60 min., 0%			
		96		Extraction & placebo			Cumulative 80%	60 min., 2%			
Pineiro et al. (2010)	BACTEC	30	Adults	Implant placement	3.30%	30 s, 15 min.	6.70%	3.30%		*St. viridans* *Neisseria cinerea*	No
		20		Implant placement preCHX	0%		0%	0%		No	No
Asi et al. (2010)	Culture	30 + 30*	Periodontitis	Modified Widman flap &amoxi	No	During surgery	13.30%			*Staphylococcus albus*, *Klebsiella*, *Ps. aeruginosa*, *St. viridans*	No
		30 + 30*		Modified Widman flap	No		46.60%				
Fine et al. (2010)	Culture	22 + 22*	Adults	Apple chewing & essential oils (2 weeks)	NR	2 min.	NR			Aerobic and anaerobic counts	NR
		22 + 22*		Apple chewing & placebo	NR		NR				NR

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 4. (continued)

Reference	Main technique	n	Patients	Intervention	Baseline	Post-intervention	1st draw	2nd draw	3rd draw	Main species	Periopathogens (n patients)
Cortelli et al. (2012)	Culture	17 + 17*	Adults	Apple chewing & essential oils (2 weeks)	NR	2 min.	NR			Aerobic and anaerobic counts	NR
		17 + 17*		Apple chewing & placebo	NR		NR				
(c)											
Murphy et al. (2006)	BACTEC	21	Periodontitis	Chewing wax 4 min.	0.00%	During, 5 min.	0.00%	0.00%		None	No
		20	Gingivitis		0.00%		0.00%	0.00%		None	No
Forner et al. (2006)	Lysis filtration	20	Periodontitis	Chewing gum 10 min.	0.00%	30 s, 10 min., 30 min.	20.00%	0.00%	5.00%	Streptococci	Pg(2), Pi(8), Fn (8)
				Manual brushing 2 min.	0.00%		5.00%	10.00%	5.00%		
				SRP	0.00%		75.00%	35.00%	10.00%		
		20	Gingivitis	Chewing gum 10 min.	0.00%		0.00%	0.00%	0.00%		
				Manual brushing 2 min.	0.00%		0.00%	0.00%	0.00%		
				SRP	0.00%		20.00%	0.00%	5.00%		
		20	Healthy	Chewing gum 10 min.	0.00%		0.00%	0.00%	0.00%		
				Manual brushing 2 min.	0.00%		0.00%	0.00%	0.00%		
				SRP	0.00%		10.00%	5.00%	0.00%		
Crasta et al. (2009)	BACTEC	30	Periodontitis	Flossing	0.00%	30 s, 10 min.	40.00%	26.70%		*St. viridans*	Aa(1),Ec(1), Pepto(4)
		30	Healthy		3.30%		41.40%	13.80%		*St. viridans*	Pm(1)
(d)											
Hutchinson et al. (1992a)	BACTEC	55	Adults	Extraction	Not done	1–2 min.	NR			Streptococci, anaerobes	*B. melaninogenicus*(5)
	Sentinel						NR			Streptococci	*B. melaninogenicus*(1)
	Isolator						NR			Streptococci	*B. melaninogenicus*(3)
Hutchinson et al. (1992b)	BACTEC	47	Adults	Third-molar surgery (GA)	Not done	1-2 min.	NR			Streptococci, anaerobes	*Fusobacterium* sp. (1)
	Oxoid Signal						NR			Streptococci, anaerobes	*Fusobacterium* sp. (3)
Lucas et al. (2002)	Lysis filtration	49	Children	Extraction	31.00%	30sec	42.90%			Streptococci	No
	BACTEC				2.10%		34.70%			Streptococci	No
Castillo et al. (2011)	Nested PCR	42	Periodontitis	Scaling	NR	Immed, 15, 30 min.	NR	NR	NR	NR	Pg(13), Aa(9), Pm(0), other
	Culture				NR		NR	NR	NR	NR	Pg(12), Aa(0), Pm(7), other

*cross-over design.

NR, not reported; GA, general anaesthesia; immed, immediate, Pepto, *Peptostreptococcus* sp.; Fuso, *Fusobacterium* sp.; Prevot, *Prevotella* sp.; Pm, *P. micra*; Pg, *P. gingivalis*; Fn, *F. nucleatum*; Pi, *P. intermedia*; Ec, *E. corrodens*; Tf, *T. forsythia*.

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 5. Description of studies evaluating the presence of periodontal pathogens in cardiovascular samples, including atheromatous lesions

Reference	Country	Patients/samples	Surgery	Vascular sample
Haraszthy et al. (2000b)	USA	50	Carotid endarterectomy	Carotid stenosis
Okuda et al. (2001)	Japan	26 test, 14 control	NR	26 atherosclerotic lesions; 14 non-diseased aorta
Stelzel et al. (2002)	Germany	26	Open heart surgery	Aortas; vascular regions
Mastragelopulos et al. (2002)	Germany	34	Carotid endarterectomy or bypass	NR
Taylor-Robinson et al. (2002)	England	36 samples	NR	Artherosclerotic major arteries
Marques da Silva et al. (2003)	Norway	49p, 53 samples	Aortic aneurysm repair	Aneurysms
Cairo et al. (2004)	Italy	26 dentate, 26 edentulous	Carotid endarterectomy	Atheromatous plaques
Ishihara et al. (2004)	Japan/USA	51	NR	Stenotic coronary artery plaques
Kurihara et al. (2004)	Japan	32	Aortic aneurysm repair	Abdominal aortic aneurysm
Marques da Silva et al. (2005)	Norway	51p, 56 lesions	Aortic aneurysm repair	Aortic aneurysms
Kozarov et al. (2005)	USA	1	Endarterectomy	Carotid
Fiehn et al. (2005)	Denmark	79 (PCR from 24)	NR	Atheromatous plaques
Padilla et al. (2006)	Chile	12 ChP	Endarterectomy	Atheromatous plaques
Kozarov et al. (2006)	USA	29p, 129 samples	Aorta, coronary arteries, carotid, femoral	Atheromatous plaques
Ott et al. (2006)	Germany	38 samples	Catheter-based atherectomy	catheter material
Zaremba et al. (2007)	Poland	20 ChP	Bypass	Coronary vessels
Aimetti et al. (2007)	Italy	33 ChP	Endarterectomy	Carotid atheromatous plaques
Romano et al. (2007)	Italy	21 ChP	Endarterectomy	Carotid atheromatous plaques
Pucar et al. (2007)	Serbia	15	Coronary artery bypass grafting surgery	Coronary arteries with atherosclerosis
Zhang et al. (2008)	China	51	Coronary artery bypass graft	Coronary atheromatous plaques
Elkaim et al. (2008)	France	22	Cardiac artery bypass	Atheromatous plaques
Gaetti-Jardim et al. (2009)	Brazil	39 ChP, 5 healthy	Endarterectomy	Atheromatous plaques from coronary arteries
Nakano et al. (2009)	Japan	223	Various	Various
Mahendra et al. (2010)	India	51 ChP	Coronary artery bypass grafting	Atheromatous plaques
Marcelino et al. (2010)	Brazil	28 test, 2 control	Endarterectomy	Atheromatous plaques
Figuero et al. (2011)	Spain	42	Endarterectomy of carotid artery	Atheromatous plaques
Aquino et al. (2011)	Brazil	30	Stent angioplasty, endarterectomy, bypass	Carotid, coronary or femoral arteries
Koren et al. (2011)	Sweden	15 atherosclerosis 15 healthy	Endarterectomy	Carotid artery
Ohki et al. (2012)	Japan	81	Primary percutaneous coronary intervention	Thrombus

NR, not reported; ChP, chronic periodontitis.

is evidence that live periodontal bacteria are present in at least some atheromas.

In vitro evidence of invasion of affected cell types

There are indisputable data that, at least, some periodontal pathogens invade human cardiovascular cells in vitro (Table 1a–d). Deshpande et al. (1998) and Dorn et al. (1999) were the first to report invasion of endothelial cells by *P. gingivalis*. Since these first reports, there have been a number of research groups who have provided details of the mechanisms of cardiovascular cell invasion by *P. gingivalis* (Dorn et al. 2000, 2002b, Rodrigues & Progulske-Fox 2005, Takahashi et al. 2006, Li et al. 2008). These have been discussed previously.

Demonstration that periodontal bacteria can promote atherosclerosis in animal models of disease

Animal models can be used to support in vitro data-based hypotheses (Graves et al. 2008) and have been used to demonstrate atherosclerotic pathology caused by periodontal pathogens. For example, *P. gingivalis* has been reported by multiple groups to accelerate atherosclerosis in murine models (Lalla et al. 2003, Gibson et al. 2004, Amar et al. 2009). In addition to murine models, rabbits with experimentally induced periodontal disease developed fatty streaks in the aorta faster than in periodontally healthy animals (Jain et al. 2003). In normocholesterolemic pigs, recurrent *P. gingivalis* bacteremia induced both aortic and coronary lesions, and *P. gingivalis* bacteremia also enhanced atherosclerosis in hypercholesterolemic pigs (Brodala et al. 2005). Thus, in addition to the identification of live periodontal organisms in human atherosclerotic plaque, in vivo experiments in a variety of animal models have provided biological plausibility that *P. gingivalis* can enhance atherogenesis.

© 2013 European Federation of Periodontology and American Academy of Periodontology

In vitro and in vivo evidence that non-invasive mutants cause significantly reduced pathology (animal model)

Mutants with significantly reduced ability to invade in vitro have also been evaluated in vivo. In contrast to the invasive wild-type strain of *P. gingivalis*, the non-invasive *fim*A deficient mutant did not accelerate atherosclerosis in *ApoE* knockout mice Gibson et al. (2004). Moreover, the *fim*A deficient mutant was less pro-atherogenic and elicited a lower level of pro-inflammatory mediators than the invasive parental strain in *ApoE* deficient mice. However, there is minimal data using other periodontal species in animal models.

Fulfill modified Koch's postulate to demonstrate that a human atheroma isolate causes disease in animal models

The final proof, fulfilling a variation of Koch's postulates, has not yet been achieved. It requires the isolation and characterization of periodontopathogens from human atheromas and the demonstration that the isolates cause atherosclerotic pathology in animal models attributable to the bacterial isolates(s). Given the success by at least one group in culturing strains of *P. gingivalis* from human atheromas (Rafferty et al. 2011), this could be accomplished in the future. An analogous approach has been used to investigate the role of an invasive strain of *Streptococcus mutans* in infectious brain aneurisms, with demonstration that a mutant deleted for the gene that allows invasion was not able to cause disease (Nakano et al. 2011).

Discussion

In this review, the evidence to assess the direct role of periodontopathogens in atherosclerosis has been evaluated considering seven proofs:

- Proof 1 – should be considered validated since the literature is convincing that oral microorganisms obtain access to the circulation.
- Proof 2 – periodontal bacteria have been identified in human atheromas by several groups using multiple detection technologies. Thus, there is little question that oral bacteria are found in human atheromas. However, visual proof of bacteria inside cells in the atheromas is still lacking. If only a minority of cells contain bacteria, transmission electron microscopy (TEM) may be too inefficient to find them. Also, there is some variation in the organisms identified in the tissues. This could be attributed to differences in techniques used or the inter-individual diversity of oral organisms able to infect atheromas. Eventually, this hypothesis could be tested in animal models but the complexity of the microbiome will complicate the models.
- Proof 3 – data using indirect means of culture that support proof 3, have been published but additional comfirmative studies are needed. In vitro, *P. gingivalis* becomes dormant or viable, but not culturable (Li et al. 2008), and in vivo viable bacteria have been cultured from atheroma homogenates following in vitro cultivation in cell lines (Rafferty et al. 2011,). It is possible that the same phenomenon occurs in vivo and that contact with "fresh" uninfected cells provides the signals to the bacteria to emerge from dormancy.
- Proof 4 – in vitro evidence of invasion of affected cell types is indisputable. During the past 15 years, there have been a series of papers reporting the invasion of cardiovascular cell types by periodontal bacteria.
- Proof 5 – it is also well documented using various animal models since the periodontal bacteria tested in these models caused increased frequency and size of atherosclerotic lesions. To the extent that animal models of atherosclerosis represent human disease, then this proof is satisfied. One goal should be the inclusion of more complex mixtures of bacteria in these models.
- Proof 6 – the invasion and disease phenotype, that is, significantly reduced disease caused by non-invasive mutants compared to the wild-type parental strain is also documented by multiple research groups. Although experiments with additional invasive mutants will prove interesting and informative, the present data confirm this proof for specific strains.
- The final proof, Proof 7, is yet to be accomplished. As atheromatous specimens become more and more difficult to obtain, we may be close to missing the window of opportunity to test this final proof.

Proposal of a model

We present a model (Fig. 2) based on what is known about the oral microbial component of atherogenesis. Both bacteremic and phagocyte-mediated avenues of bacterial delivery to the site of inflammation are proposed. Bacteremia-derived bacteria invade the endothelial layer and further spread into deeper tissue (left). The activation of the infected endothelia results in the release of pro-inflammatory chemokines [such as monocyte chemotactic protein (MCP-1)] in the lumen, resulting in activation of blood monocytes (MN) and macrophages (MΦ) promoting their adhesion and diapedesis. In addition, transmigrated leucocytes (in the centre) can harbour internalized viable bacteria, which represents the second avenue for systemic bacterial dissemination to distant sites. The bacteria adhere to the endothelial cells, enter and usurp the endothelial cell processes for trafficking, at which point they may become uncultivable (red into green). Their internalization by phagocytes or interactions with uninfected cells can reactivate them (from green to red). Atheromas can grow due to macrophage-secreted growth factors resulting in smooth muscle cell proliferation. Bacteria are also released upon host cell death (depicted at right) to re-infect additional cells.

Significance of strain

Throughout the microbial world, there are strain differences within a species and these differences sometimes influence virulence. We propose that such is the case for periodontopathogenic species in relation to cardiovascular diseases. There are multiple examples cited in this review that illustrate phenotypic differences among strains of a

© 2013 European Federation of Periodontology and American Academy of Periodontology

Table 6. Results of studies evaluating the presence of periodontal pathogens in cardiovascular samples, including atheromatous lesions

Reference	Periodontal exam	Subgingival samples		Universal	*Aa*	*Pg*	*Td*	*Tf*	*Pi*	*Fn*	*Ec*	*Cr*
Haraszthy et al. (2000b)	No	No	Specific PCR	72%	18%	26%		30%	14%			
Okuda et al. (2001)	No	No	Specific PCR	NR	0%	0%	23.10%	0%				0%
Stelzel et al. (2002)	No	No	Specific PCR	88.5%	0%	15.4%						
Mastra gelopulos et al. (2002)	No	No	Specific PCR	59%	NR	NR	NR					
Taylor-Robinson et al. (2002)	NR	NR	Specific PCR	31.2%	21.9%	9.4%						
Marques da Silva et al. (2003)	No	No	Specific PCR		7.1%	0%		0%				
Cairo et al. (2004)	Yes	Yes	Specific PCR		0%	0%		0%	0%	0%		
Ishihara et al. (2004)	Yes	Yes	Specific PCR		23.3%	21.6%	23.5%	5.9%				15.7%
Kurihara et al. (2004)	Yes	Yes	Specific PCR		0%	85%	63%	22%	31%			45%
Marques da Silva et al. (2005)	No	No	Specific PCR	89.2%	7.1%	0%	0%	0%				
Kozarov et al. (2005)	No	No	Cell-culture		100%	100%						
Fiehn et al. (2005)	No	No	Nested PCR	100%	0%	4.17%		0%	100%			0%
Padilla et al. (2006)	Yes	Yes	Culture		16.67%	0%	0%	0%	0%	0%	0%	0%
Kozarov et al. (2006)	No	No	Specific PCR		40/55.5%	18.3/ 88.8%	19.1/ 33.3%	15/ 22.2%	15/ 77.7%		17.5/ 22.2%	
Zaremba et al. (2007)	Yes	Yes	Hybrid-ization		5.00%	50.0%	30.0%	25.0%	15.0%	20.0%	15.0%	20.0%
Aimetti et al. (2007)	Yes	Yes	Nested PCR	94%	0%	0%	0%	0%	0%			
Romano et al. (2007)	Yes	Yes	Hybrid-ization		0%	0%	0%	0%	0%			
Pucar et al. (2007)	No	No	Specific PCR		26.7%	53.3%		13.3%	33.3%			
Zhang et al. (2008)	Yes	Yes	Specific PCR		0%	33%		31%	18%	12%		
Elkaim et al. (2008)	Yes	Yes	Hybrid-ization		54.5%	54.5–72.7%					22.7%	27.3%
Gaetti-Jardim et al. (2009)	Yes	No	Real-time PCR		46.2%	53.8%		25.6%	59%	0%		
Nakano et al. (2009)	No	Yes	Specific PCR		30-35%	15-20%	15-20%					5%
Mahendra et al. (2010)	Yes	Yes	Specific PCR			45.1%	49.01%					21.5%
Figuero et al. (2011)	Yes	No	Nested PCR		66.7%	78.6%		61.9		50%	54.8%	9.5%
Aquino et al. (2011)	Yes	No	Specific PCR	13%	0%	0%	0%					
Ohki et al. (2012)	No	No	Specific PCR		21%	3.7%	2.5%	0.0%	0.0%			

NR, not reported; ChP, chronic periodontitis, *Aa*, *A. actinomycetemcomitans; Pg, P. gingivalis; Td, T. denticola; Tf, T. forsythia; Pm, P. micra; Pi, P. intermedia; Fn, F. nucleatum; Ec, E. corrodens; Cr, C. rectus.*

© 2013 European Federation of Periodontology and American Academy of Periodontology

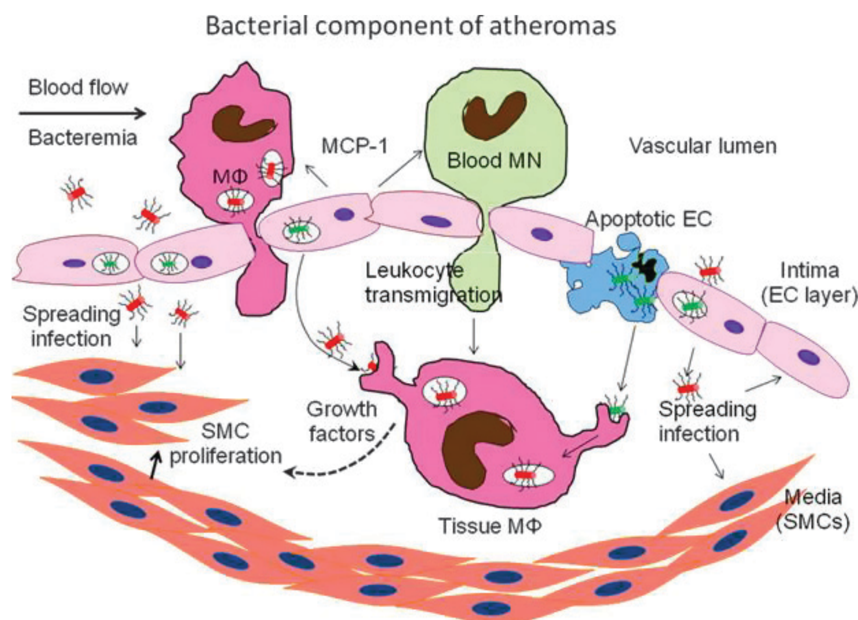

Fig 2. Model representing what is now known about the microbial component of atherogenesis. Both bacteremic and phagocyte-mediated avenues of bacterial delivery to the site of inflammation are proposed. Bacteremia-related bacteria (left) are shown invading the endothelial layer and further spreading into deeper tissue. Activation of infected endothelia are represented with the release of pro-inflammatory chemokines (such as MCP-1) in the lumen, activating blood monocytes (MN) and macrophages (MΦ) and promoting their adhesion and diapedesis. Transmigrating leucocytes (in the centre) can harbour internalized viable bacteria, which represents the second avenue for systemic bacterial dissemination to distant sites. The internalization of bacteria can switch them into uncultivable state (red into green), while their internalization by phagocytes can reactivate them (from green to red). Atheromas can grow due to macrophage-secreted growth factors-mediated smooth muscle cell proliferation. Bacteria are also released upon host cell death (depicted at right). MN, monocyte. MΦ, macrophage with internalized bacteria. EC, endothelial cell. SMC, smooth muscle cell. Apoptotic EC, apoptotic endothelial cell releasing intracellular bacteria.

particular species. For example, Brissette & Fives-Taylor (1999) reported the invasive heterogeneity of *A. actinomycetemecomitans* strains. Also, as discussed above, only certain *P. gingivalis* strains induce autophagy in endothelial cells and strains vary in the number of repeats in the HagA adhesin. In addition, we have tested over 20 *P. gingivalis* strains for the ability to invade various cell types (Dorn et al. 2000). Our results revealed a wide ability among strains to invade cardiovascular cells with strains grouped into categories including high invaders, moderate invaders and non-invaders. Thus, it is likely that different strains of *P. gingivalis* induce varying responses in promoting endothelial dysfunction. For example, *P. gingivalis* 381 (fimbriae type I) induces gene expression of GroA, GroE, IL-6, IL-8, vascular cell adhesion molecule (VCAM)-1 and endothelial leukocyte adhesion molecule (ELAM)-1 in HCAECs, which is a fimbriae dependent phenomenon (Chou et al. 2005) that is mediated through engagement of TLRs (Yumoto et al. 2005). Conversely, *P. gingivalis* strain W83, which is capsule positive but does not express fimbriae, induces moderate activation of TLR2 and an attenuated inflammatory response in HCAEC when compared to 381 (Rodrigues et al. 2012). Similarly, capsule positive A7436, which expresses type IV fimbriae, also induces a moderate inflammatory response in HCAEC (unpublished data). Interestingly, both W83 and A7436 can accelerate atherosclerosis in ApoE-null mice (Li et al. 2002, Maekawa et al. 2011), suggesting that periodontal bacteria can promote atherosclerosis through other mechanisms that may not involve profound activation of endothelial cells. Interestingly, similar studies with *S. mutans* strains have demonstrated that 10-15% of strains can invade cardiovascular endothelial cells and that an invasive strain caused significant pathology compared to control mice in an ApoE-/- mouse model of accelerated atherosclerosis (Kesavalu et al. 2012).

Consequently, based on the available information, we suggest that only specific strains of invasive periodontopathogens play a role in infections of cardiovascular tissues. The significance of this is that individuals harbouring these strains may be at a higher risk for cardiovascular diseases (Nakano et al. 2007). Consequently, the chair-side detection of pro-atherogenic strains may be a goal of the future.

Conclusions

In conclusion, considering the evidence for the postulates provided here, there is strong, but not yet conclusive, evidence for invasion of human cardiovascular cells by periodontal bacterial species as one mechanism of atherosclerosis. Since Koch's Postulates cannot be applied to humans, in this case we suggest a modified Koch's postulate be tested if, and when appropriate strains and mutants of those strains are obtained and constructed. Thus, additional work is required to confirm or dispute this aetiology. This additional work would not only lead to better understanding of the mechanisms of infectious atherosclerosis but also may be a key to the design of novel and effective treatment and preventive approaches.

Acknowledgements

Elena Figuero, María José Marín and Denisse Hernández (University Complutense of Madrid) are sincerely acknowledged for their collaboration in the topics of bacteremia and bacterial detection in atheromatous plaques. The authors thank Jacob Burks (University of Florida) for his assistance in preparing the manuscript.

© 2013 European Federation of Periodontology and American Academy of Periodontology

References

Abranches, J., Zeng, L., Belanger, M., Rodrigues, P. H., Simpson-Haidaris, P. J., Akin, D., Dunn, W. A. Jr, Progulske-Fox, A. & Burne, R. A. (2009) Invasion of human coronary artery endothelial cells by *Streptococcus mutans* OMZ175. *Oral Microbiology and Immunology* **24**, 141–145.

Aimetti, M., Romano, F. & Nessi, F. (2007) Microbiologic analysis of periodontal pockets and carotid atheromatous plaques in advanced chronic periodontitis patients. *Journal of Periodontology* **78**, 1718–1723.

Amano, A. (2010) Bacterial adhesins to host components in periodontitis. *Periodontology 2000* **52**, 12–37.

Amar, S., Wu, S. C. & Madan, M. (2009) Is *Porphyromonas gingivalis* cell invasion required for atherogenesis? Pharmacotherapeutic implications. *Journal of Immunology* **182**, 1584–1592.

Anand, P. K., Tait, S. W., Lamkanfi, M., Amer, A. O., Nunez, G., Green, D. R. & Kanneganti, T. D. (2011) TLR2 and RIP2 pathways mediate autophagy of *Listeria monocytogenes* via ERK activation. *Journal of Biological Chemistry* **286**, 42981–42991.

Aquino, A. R., Lima, K. C., Paiva, M. S., Rocas, I. N. & Siqueira, J. F. Jr (2011) Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi. *Journal of Periodontal Research* **46**, 303–309.

Arce, R. M., Diaz, P. I., Barros, S. P., Galloway, P., Bobetsis, Y., Threadgill, D. & Offenbacher, S. (2010) Characterization of the invasive and inflammatory traits of oral *Campylobacter rectus* in a murine model of fetoplacental growth restriction and in trophoblast cultures. *Journal of Reproductive Immunology* **84**, 145–153.

Arirachakaran, P., Apinhasmit, W., Paungmalit, P., Jeramethakul, P., Rerkyen, P. & Mahanonda, R. (2012) Infection of human gingival fibroblasts with *Aggregatibacter actinomycetemcomitans*: an in vitro study. *Archives of Oral Biology* **57**, 964–972.

Aruni, A. W., Roy, F. & Fletcher, H. M. (2011) *Filifactor alocis* has virulence attributes that can enhance its persistence under oxidative stress conditions and mediate invasion of epithelial cells by *Porphyromonas gingivalis*. *Infection and Immunity* **79**, 3872–3886.

Asakawa, R., Komatsuzawa, H., Kawai, T., Yamada, S., Goncalves, R. B., Izumi, S., Fujiwara, T., Nakano, Y., Suzuki, N., Uchida, Y., Ouhara, K., Shiba, H., Taubman, M. A., Kurihara, H. & Sugai, M. (2003) Outer membrane protein 100, a versatile virulence factor of *Actinobacillus actinomycetemcomitans*. *Molecular Microbiology* **50**, 1125–1139.

Asi, K. S., Gill, A. S. & Mahajan, S. (2010) Postoperative bacteremia in periodontal flap surgery, with and without prophylactic antibiotic administration: a comparative study. *Journal of Indian Society Periodontology* **14**, 18–22.

Bahrani-Mougeot, F. K., Paster, B. J., Coleman, S., Ashar, J., Barbuto, S. & Lockhart, P. B. (2008) Diverse and novel oral bacterial species in blood following dental procedures. *Journal of Clinical Microbiology* **46**, 2129–2132.

Bartruff, J. B., Yukna, R. A. & Layman, D. L. (2005) Outer membrane vesicles from *Porphyromonas gingivalis* affect the growth and function of cultured human gingival fibroblasts and umbilical vein endothelial cells. *Journal of Periodontology* **76**, 972–979.

Belanger, M., Kozarov, E., Song, H., Whitlock, J. & Progulske-Fox, A. (2012) Both the unique and repeat regions of the *Porphyromonas gingivalis* hemagglutin A are involved in adhesion and invasion of host cells. *Anaerobe* **18**, 128–134.

Bernales, S., McDonald, K. L. & Walter, P. (2006) Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biology* **4**, e423.

Braunersreuther, V., Mach, F. & Steffens, S. (2007) The specific role of chemokines in atherosclerosis. *Thrombosis and Haemostasis* **97**, 714–721.

Brennan, M. T., Kent, M. L., Fox, P. C., Norton, H. J. & Lockhart, P. B. (2007) The impact of oral disease and nonsurgical treatment on bacteremia in children. *Journal of the American Dental Association* **138**, 80–85.

Brissette, C. A. & Fives-Taylor, P. M. (1999) *Actinobacillus actinomycetemcomitans* may utilize either actin-dependent or actin-independent mechanisms of invasion. *Oral Microbiology and Immunology* **14**, 137–142.

Brodala, N., Merricks, E. P., Bellinger, D. A., Damrongsri, D., Offenbacher, S., Beck, J., Madianos, P., Sotres, D., Chang, Y. L., Koch, G. & Nichols, T. C. (2005) *Porphyromonas gingivalis* bacteremia induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**, 1446–1451.

Buhlin, K., Hultin, M., Norderyd, O., Persson, L., Pockley, A. G., Rabe, P., Klinge, B. & Gustafsson, A. (2009) Risk factors for atherosclerosis in cases with severe periodontitis. *Journal of Clinical Periodontology* **36**, 541–549.

Cairo, F., Gaeta, C., Dorigo, W., Oggioni, M. R., Pratesi, C., Pini Prato, G. P. & Pozzi, G. (2004) Periodontal pathogens in atheromatous plaques. A controlled clinical and laboratory trial. *Journal of Periodontal Research* **39**, 442–446.

Cao, S. L., Progulske-Fox, A., Hillman, J. D. & Handfield, M. (2004) *In vivo* induced antigenic determinants of *Actinobacillus actinomycetemcomitans*. *FEMS Microbiology Letters* **237**, 97–103.

Carrion, J., Scisci, E., Miles, B., Sabino, G. J., Zeituni, A. E., Gu, Y., Bear, A., Genco, C. A., Brown, D. L. & Cutler, C. W. (2012) Microbial carriage state of peripheral blood dendritic cells (DCs) in chronic periodontitis influences DC differentiation, atherogenic potential. *Journal of Immunology* **189**, 3178–3187.

Castillo, D. M., Sanchez-Beltran, M. C., Castellanos, J. E., Sanz, I., Mayorga-Fayad, I., Sanz, M. & Lafaurie, G. I. (2011) Detection of specific periodontal microorganisms from bacteraemia samples after periodontal therapy using molecular-based diagnostics. *Journal of Clinical Periodontology* **38**, 418–427.

Chalmers, N. I., Palmer, R. J.. Jr, Cisar, J. O. & Kolenbrander, P. E. (2008) Characterization of a Streptococcus sp.-Veillonella sp. community micromanipulated from dental plaque. *Journal of Bacteriology* **190**, 8145–8154.

Chavakis, T., Wiechmann, K., Preissner, K. T. & Herrmann, M. (2005) *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. *Thrombosis and Haemostasis* **94**, 278–285.

Cherry, M., Daly, C. G., Mitchell, D. & Highfield, J. (2007) Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. *Journal of Clinical Periodontology* **34**, 148–155.

Chou, H. H., Yumoto, H., Davey, M., Takahashi, Y., Miyamoto, T., Gibson, F. C. 3rd & Genco, C. A. (2005) *Porphyromonas gingivalis* fimbria-dependent activation of inflammatory genes in human aortic endothelial cells. *Infection and Immunity* **73**, 5367–5378.

Christersson, L. A., Albini, B., Zambon, J. J., Wikesjo, U. M. & Genco, R. J. (1987a) Tissue localization of *Actinobacillus actinomycetemcomitans* in human periodontitis. I. Light, immunofluorescence and electron microscopic studies. *Journal of Periodontology* **58**, 529–539.

Christersson, L. A., Wikesjo, U. M., Albini, B., Zambon, J. J. & Genco, R. J. (1987b) Tissue localization of *Actinobacillus actinomycetemcomitans* in human periodontitis. II. Correlation between immunofluorescence and culture techniques. *Journal of Periodontology* **58**, 540–545.

Colombo, A. V., da Silva, C. M., Haffajee, A. & Colombo, A. P. (2007) Identification of intracellular oral species within human crevicular epithelial cells from subjects with chronic periodontitis by fluorescence in situ hybridization. *Journal of Periodontal Research* **42**, 236–243.

Colombo, A. V., Silva, C. M., Haffajee, A. & Colombo, A. P. (2006) Identification of oral bacteria associated with crevicular epithelial cells from chronic periodontitis lesions. *Journal of Medical Microbiology* **55**, 609–615.

Cortelli, J. R., Cogo, K., Aquino, D. R., Cortelli, S. C., Ricci-Nittel, D., Zhang, P. & Araujo, M. W. (2012) Validation of the anti-bacteremic efficacy of an essential oil rinse in a Brazilian population: a cross-over study. *Brazilian Oral Research* **26**, 478–484.

Crasta, K., Daly, C. G., Mitchell, D., Curtis, B., Stewart, D. & Heitz-Mayfield, L. J. (2009) Bacteraemia due to dental flossing. *Journal of Clinical Periodontology* **36**, 323–332.

Darenfed, H., Grenier, D. & Mayrand, D. (1999) Acquisition of plasmin activity by *Fusobacterium nucleatum* subsp. *nucleatum* and potential contribution to tissue destruction during periodontitis. *Infection and Immunity* **67**, 6439–6444.

Deniset, J. F. & Pierce, G. N. (2010) Possibilities for therapeutic interventions in disrupting Chlamydophila pneumoniae involvement in atherosclerosis. *Fundamental & Clinical Pharmacology* **24**, 607–617.

Deretic, V. (2011) Autophagy in immunity and cell-autonomous defense against intracellular microbes. *Immunological Reviews* **240**, 92–104.

Deretic, V. & Levine, B. (2009) Autophagy, immunity, and microbial adaptations. *Cell Host & Microbe* **5**, 527–549.

Deshpande, R. G., Khan, M. B. & Genco, C. A. (1998) Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. *Infection and Immunity* **66**, 5337–5343.

DiRienzo, J. M., Song, M., Wan, L. S. & Ellen, R. P. (2002) Kinetics of KB and HEp-2 cell responses to an invasive, cytolethal distending toxin-producing strain of Actinobacillus actinomycetemcomitans. *Oral Microbiology and Immunology* **17**, 245–251.

Diz Dios, P., Tomas Carmona, I., Limeres Posse, J., Medina Henriquez, J., Fernandez Feijoo, J. & Alvarez Fernandez, M. (2006) Comparative efficacies of amoxicillin, clindamycin, and moxifloxacin in prevention of bacteremia following dental extractions. *Antimicrobial Agents and Chemotherapy* **50**, 2996–3002.

© 2013 European Federation of Periodontology and American Academy of Periodontology

Dolgilevich, S., Rafferty, B., Luchinskaya, D. & Kozarov, E. (2011) Genomic comparison of invasive and rare non-invasive strains reveals *Porphyromonas gingivalis* genetic polymorphisms. *Journal of Oral Microbiology* **3**, 5764–5774.

Dorn, B. R., Burks, J. N., Seifert, K. N. & Progulske-Fox, A. (2000) Invasion of endothelial and epithelial cells by strains of *Porphyromonas gingivalis*. *FEMS Microbiology Letters* **187**, 139–144.

Dorn, B. R., Dunn, W. A. Jr & Progulske-Fox, A. (1999) Invasion of human coronary artery cells by periodontal pathogens. *Infection and Immunity* **67**, 5792–5798.

Dorn, B. R., Dunn, W. A. Jr & Progulske-Fox, A. (2001) *Porphyromonas gingivalis* traffics to autophagosomes in human coronary artery endothelial cells. *Infection and Immunity* **69**, 5698–5708.

Dorn, B. R., Dunn, W. A. Jr & Progulske-Fox, A. (2002a) Bacterial interactions with the autophagic pathway. *Cellular Microbiology* **4**, 1–10.

Dorn, B. R., Harris, L. J., Wujick, C. T., Vertucci, F. J. & Progulske-Fox, A. (2002b) Invasion of vascular cells in vitro by *Porphyromonas endodontalis*. *International Endododontic Journal* **35**, 366–371.

Dorn, B. R., Leung, K. & Progulske-Fox, A. (1998) Invasion of human oral epithelial cells by *Prevotella intermedia*. *Infection and Immunity* **66**, 6054–6057.

Edwards, A. M., Grossman, T. J. & Rudney, J. D. (2006) *Fusobacterium nucleatum* transports noninvasive *Streptococcus cristatus* into human epithelial cells. *Infection and Immunity* **74**, 654–662.

Eick, S., Reissmann, A., Rodel, J., Schmidt, K. H. & Pfister, W. (2006) *Porphyromonas gingivalis* survives within KB cells and modulates inflammatory response. *Oral Microbiology and Immunology* **21** (4), 231–237.

Elkaim, R., Dahan, M., Kocgozlu, L., Werner, S., Kanter, D., Kretz, J. G. & Tenenbaum, H. (2008) Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: a preliminary study. *Journal of Periodontal Research* **43**, 224–231.

Epstein, S. E., Zhu, J., Najafi, A. H. & Burnett, M. S. (2009) Insights into the role of infection in atherogenesis and in plaque rupture. *Circulation* **119**, 3133–3141.

Fedele, S., Sabbah, W., Donos, N., Porter, S. & D'Aiuto, F. (2011) Common oral mucosal diseases, systemic inflammation, and cardiovascular diseases in a large cross-sectional US survey. *American Heart Journal* **161**, 344–350.

Fiehn, N. E., Larsen, T., Christiansen, N., Holmstrup, P. & Schroeder, T. V. (2005) Identification of periodontal pathogens in atherosclerotic vessels. *Journal of Periodontology* **76**, 731–736.

Figuero, E., Sanchez-Beltran, M., Cuesta-Frechoso, S., Tejerina, J. M., del Castro, J. A., Gutierrez, J. M., Herrera, D. & Sanz, M. (2011) Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *Journal of Periodontology* **82**, 1469–1477.

Fine, D. H., Furgang, D., McKiernan, M., Tereski-Bischio, D., Ricci-Nittel, D., Zhang, P. & Araujo, M. W. (2010) An investigation of the effect of an essential oil mouthrinse on induced bacteraemia: a pilot study. *Journal of Clinical Periodontology* **37**, 840–847.

Fitzpatrick, R. E., Wijeyewickrema, L. C. & Pike, R. N. (2009) The gingipains: scissors and glue of the periodontal pathogen, *Porphyromonas gingivalis*. *Future Microbiology* **4**, 471–487.

Fives-Taylor, P. M., Meyer, D. H., Mintz, K. P. & Brissette, C. (1999) Virulence factors of *Actinobacillus actinomycetemcomitans*. *Periodontology* **2000** (20), 136–167.

Forner, L., Larsen, T., Kilian, M. & Holmstrup, P. (2006) Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *Journal of Clinical Periodontology* **33**, 401–407.

Gaetti-Jardim, E. Jr, Marcelino, S. L., Feitosa, A. C., Romito, G. A. & Avila-Campos, M. J. (2009) Quantitative detection of periodontopathic bacteria in atherosclerotic plaques from coronary arteries. *Journal of Medical Microbiology* **58**, 1568–1575.

Gibson, F. C. 3rd, Hong, C., Chou, H. H., Yumoto, H., Chen, J., Lien, E., Wong, J. & Genco, C. A. (2004) Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **109**, 2801–2806.

Graves, D. T., Fine, D., Teng, Y. T., Van Dyke, T. E. & Hajishengallis, G. (2008) The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. *Journal of Clinical Periodontology* **35**, 89–105.

Hajishengallis, G., Wang, M., Harokopakis, E., Triantafilou, M. & Triantafilou, K. (2006) *Porphyromonas gingivalis* fimbriae proactively modulate beta2 integrin adhesive activity and promote binding to and internalization by macrophages. *Infection and Immunity* **74**, 5658–5666.

Hajishengallis, G., Wang, M., Liang, S., Triantafilou, M. & Triantafilou, K. (2008) Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. *Proceedings of the National Academy of Science USA* **105**, 13532–13537.

Han, Y. W., Shi, W., Huang, G. T., Kinder Haake, S., Park, N. H., Kuramitsu, H. & Genco, R. J. (2000) Interactions between periodontal bacteria and human oral epithelial cells: *Fusobacterium nucleatum* adheres to and invades epithelial cells. *Infection and Immunity* **68**, 3140–3146.

Haraszthy, V. I., Hariharan, G., Tinoco, E. M., Cortelli, J. R., Lally, E. T., Davis, E. & Zambon, J. J. (2000a) Evidence for the role of highly leukotoxic *Actinobacillus actinomycetemcomitans* in the pathogenesis of localized juvenile and other forms of early-onset periodontitis. *Journal of Periodontology* **71**, 912–922.

Haraszthy, V. I., Zambon, J. J., Trevisan, M., Zeid, M. & Genco, R. J. (2000b) Identification of periodontal pathogens in atheromatous plaques. *Journal of Periodontal Research* **71**, 1554–1560.

Harokopakis, E., Albzreh, M. H., Martin, M. H. & Hajishengallis, G. (2006) TLR2 transmodulates monocyte adhesion and transmigration via Rac1- and PI3K-mediated inside-out signaling in response to *Porphyromonas gingivalis* fimbriae. *Journal of Immunology* **176**, 7645–7656.

Heimdahl, A., Hall, G., Hedberg, M., Sandberg, H., Soder, P. O., Tuner, K. & Nord, C. E. (1990) Detection and quantitation by lysis-filtration of bacteremia after different oral surgical procedures. *Journal of Clinical Microbiology* **28**, 2205–2209.

Hertzen, E., Johansson, L., Wallin, R., Schmidt, H., Kroll, M., Rehn, A. P., Kotb, M., Morgelin, M. & Norrby-Teglund, A. (2010) M1 protein-dependent intracellular trafficking promotes persistence and replication of *Streptococcus pyogenes* in macrophages. *Journal of Innate Immunity* **2**, 534–545.

Higashi, Y., Goto, C., Hidaka, T., Soga, J., Nakamura, S., Fujii, Y., Hata, T., Idei, N., Fujimura, N., Chayama, K., Kihara, Y. & Taguchi, A. (2009) Oral infection-inflammatory pathway, periodontitis, is a risk factor for endothelial dysfunction in patients with coronary artery disease. *Atherosclerosis* **206**, 604–610.

Hutchinson, N. A., Shanson, D. C. & Malins, T. (1992a) Clinical comparison of Sentinel, a novel blood culture system, with radiometric Bactec 460 and Isolator 10 in the detection of streptococcal and anaerobic bacteraemias. *Journal of Clinical Pathology* **45**, 475–478.

Hutchinson, N. A., Thomas, F. D. & Shanson, D. C. (1992b) The clinical comparison of Oxoid Signal with Bactec blood culture systems for the detection of streptococcal and anaerobic bacteraemias. *Journal of Medical Microbiology* **37**, 410–412.

Hybiske, K. & Stephens, R. S. (2007) Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. *Proceedings of the Natoinal Academy of Science USA* **104**, 11430–11435.

Ikegami, A., Chung, P. & Han, Y. W. (2009) Complementation of the fadA mutation in *Fusobacterium nucleatum* demonstrates that the surface-exposed adhesin promotes cellular invasion and placental colonization. *Infection and Immunity* **77**, 3075–3079.

Inagaki, S., Onishi, S., Kuramitsu, H. K. & Sharma, A. (2006) *Porphyromonas gingivalis* vesicles enhance attachment, and the leucine-rich repeat BspA protein is required for invasion of epithelial cells by *Tannerella forsythia*. *Infection and Immunity* **74**, 5023–5028.

Ishihara, K., Nabuchi, A., Ito, R., Miyachi, K., Kuramitsu, H. K. & Okuda, K. (2004) Correlation between detection rates of periodontopathic bacterial DNA in coronary stenotic artery plaque [corrected] and in dental plaque samples. *Journal of Clinical Microbiology* **42**, 1313–1315.

Iyer, D., Anaya-Bergman, C., Jones, K., Yanamandra, S., Sengupta, D., Miyazaki, H. & Lewis, J. P. (2010) AdpC is a Prevotella intermedia 17 leucine-rich repeat internalin-like protein. *Infection and Immunity* **78**, 2385–2396.

Jain, A., Batista, E. L. Jr, Serhan, C., Stahl, G. L. & Van Dyke, T. E. (2003) Role for periodontitis in the progression of lipid deposition in an animal model. *Infection and Immunity* **71**, 6012–6018.

Ji, S., Shin, J. E., Kim, Y. C. & Choi, Y. (2010) Intracellular degradation of *Fusobacterium nucleatum* in human gingival epithelial cells. *Molecules and Cells* **30**, 519–526.

Johnson, J. D., Chen, R., Lenton, P. A., Zhang, G., Hinrichs, J. E. & Rudney, J. D. (2008) Persistence of extracrevicular bacterial reservoirs after treatment of aggressive periodontitis. *Journal of Periodontology* **79**, 2305–2312.

Katz, J., Chaushu, G. & Sharabi, Y. (2001) On the association between hypercholesterolemia, cardiovascular disease and severe periodontal disease. *Journal of Clinical Periodontology* **28**, 865–868.

Kebschull, M., Demmer, R. T. & Papapanou, P. N. (2010) "Gum bug, leave my heart alone!"–epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. *Journal of Dental Research* **89**, 879–902.

Keizer, H. G. (2012) The "Mevalonate hypothesis": a cholesterol-independent alternative for the etiology of atherosclerosis. *Lipids in Health and Disease* **11**, 149.

Kesavalu, L., Lucas, A. R., Verma, R. K., Liu, L., Dai, E., Sampson, E. & Progulske-Fox, A.

© 2013 European Federation of Periodontology and American Academy of Periodontology

(2012) Increased atherogenesis during *Streptococcus mutans* infection in ApoE-null mice. *Journal of Dental Research* **91**, 255–260.

Khan, M. J., Rizwan Alam, M., Waldeck-Weiermair, M., Karsten, F., Groschner, L., Riederer, M., Hallstrom, S., Rockenfeller, P., Konya, V., Heinemann, A., Madeo, F., Graier, W. F. & Malli, R. (2012) Inhibition of Autophagy Rescues Palmitic Acid-induced Necroptosis of Endothelial Cells. *Journal of Biological Chemistry* **287**, 21110–21120.

Kinane, D. F., Riggio, M. P., Walker, K. F., MacKenzie, D. & Shearer, B. (2005) Bacteraemia following periodontal procedures. *Journal of Clinical Periodontology* **32**, 708–713.

Kinane, J. A., Benakanakere, M. R., Zhao, J., Hosur, K. B. & Kinane, D. F. (2012) *Porphyromonas gingivalis* influences actin degradation within epithelial cells during invasion and apoptosis. *Cellular Microbiology* **14**, 1085–1096.

Kirschbaum, M., Schultze-Mosgau, S., Pfister, W. & Eick, S. (2010) Mixture of periodontopathogenic bacteria influences interaction with KB cells. *Anaerobe* **16**, 461–468.

Kolattukudy, P. E. & Niu, J. (2012) Inflammation, endoplasmic reticulum stress, autophagy, and the monocyte chemoattractant protein-1/CCR2 pathway. *Circulation Research* **110**, 174–189.

Komatsuzawa, H., Asakawa, R., Kawai, T., Ochiai, K., Fujiwara, T., Taubman, M. A., Ohara, M., Kurihara, H. & Sugai, M. (2002) Identification of six major outer membrane proteins from *Actinobacillus actinomycetemcomitans*. *Gene* **288**, 195–201.

Koren, O., Spor, A., Felin, J., Fak, F., Stombaugh, J., Tremaroli, V., Behre, C. J., Knight, R., Fagerberg, B., Ley, R. E. & Backhed, F. (2011) Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 4592–4598.

Kozarov, E., Dorn, B., Shelburne, C., Dunn, W. & Progulske-Fox, A. (2005) Human atherosclerotic plaque contains viable invasive *Porphyromonas gingivalis* and *Actinobacillus actinomy cetemcomitans*. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**, e17–e18.

Kozarov, E., Sweier, D., Shelburne, C., Progulske-Fox, A. & Lopatin, D. (2006) Detection of bacterial DNA in atheromatous plaques by quantitative PCR. *Microbes and infection/Institut Pasteur* **8**, 687–693.

Kozarov, E., Whitlock, J., Dong, H., Carrasco, E. & Progulske-Fox, A. (1998) The number of direct repeats in *hagA* is variable among *Porphyromonas gingivalis* strains. *Infection and Immunity* **66**, 4721–4725.

Kurihara, N., Inoue, Y., Iwai, T., Umeda, M., Huang, Y. & Ishikawa, I. (2004) Detection and localization of periodontopathic bacteria in abdominal aortic aneurysms. *European Journal of Vascular and Endovascular Surgery* **28**, 553–558.

Lafaurie, G. I., Mayorga-Fayad, I., Torres, M. F., Castillo, D. M., Aya, M. R., Baron, A. & Hurtado, P. A. (2007) Periodontopathic microorganisms in peripheric blood after scaling and root planing. *Journal of Clinical Periodontology* **34**, 873–879.

Lalla, E., Lamster, I. B., Hofmann, M. A., Bucciarelli, L., Jerud, A. P., Tucker, S., Lu, Y., Papapanou, P. N. & Schmidt, A. M. (2003) Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **23**, 1405–1411.

Larocca, T. J., Henson, G. D., Thorburn, A., Sindler, A. L., Pierce, G. L. & Seals, D. R. (2012) Translational evidence that impaired autophagy contributes to arterial ageing. *Journal of Physiology* **590**, 3305–3316.

Leivadaros, E., van der Velden, U., Bizzarro, S., ten Heggeler, J. M., Gerdes, V. E., Hoek, F. J., Nagy, T. O., Scholma, J., Bakker, S. J., Gans, R. O., ten Cate, H. & Loos, B. G. (2005) A pilot study into measurements of markers of atherosclerosis in periodontitis. *Journal of Periodontology* **76**, 121–128.

Lepine, G., Caudry, S., DiRienzo, J. M. & Ellen, R. P. (1998) Epithelial cell invasion by *Actinobacillus actinomycetemcomitans* strains from restriction fragment-length polymorphism groups associated with juvenile periodontitis or carrier status. *Oral Microbiology and Immunology* **13**, 341–347.

Leung, N. M., Chen, R. & Rudney, J. D. (2006) Oral bacteria in plaque and invading buccal cells of young orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopedics* **130** (698), e611–e698.

Li, L., Matevski, D., Aspiras, M., Ellen, R. P. & Lepine, G. (2004) Two epithelial cell invasion-related loci of the oral pathogen *Actinobacillus actinomycetemcomitans*. *Oral Microbiologyogy and Immunology* **19**, 16–25.

Li, L., Messas, E., Batista, E. L. Jr, Levine, R. A. & Amar, S. (2002) *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation* **105**, 861–867.

Li, L., Michel, R., Cohen, J., DeCarlo, A. & Kozarov, E. (2008) Intracellular survival and vascular cell-to-cell transmission of *Porphyromonas gingivalis*. *BMC Microbiology* **8**, 26–36.

Liang, S., Krauss, J. L., Domon, H., McIntosh, M. L., Hosur, K. B., Qu, H., Li, F., Tzekou, A., Lambris, J. D. & Hajishengallis, G. (2011) The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss. *Journal of Immunology* **186**, 869–877.

Liao, X., Sluimer, J. C., Wang, Y., Subramanian, M., Brown, K., Pattison, J. S., Robbins, J., Martinez, J. & Tabas, I. (2012) Macrophage autophagy plays a protective role in advanced atherosclerosis. *Cellular Metabolism* **15**, 545–553.

Libby, P., Ridker, P. M. & Maseri, A. (2002) Inflammation and atherosclerosis. *Circulation* **105**, 1135–1143.

Lockhart, P. B., Brennan, M. T., Sasser, H. C., Fox, P. C., Paster, B. J. & Bahrani-Mougeot, F. K. (2008) Bacteremia associated with toothbrushing and dental extraction. *Circulation* **117**, 3118–3125.

Lockhart, P. B., Brennan, M. T., Thornhill, M., Michalowicz, B. S., Noll, J., Bahrani-Mougeot, F. K. & Sasser, H. C. (2009) Poor oral hygiene as a risk factor for infective endocarditis-related bacteremia. *Journal of the American Dental Association* **140**, 1238–1244.

Lucas, V. S., Gafan, G., Dewhurst, S. & Roberts, G. J. (2008) Prevalence, intensity and nature of bacteraemia after toothbrushing. *Journal of Dentistry* **36**, 481–487.

Lucas, V. S., Lytra, V., Hassan, T., Tatham, H., Wilson, M. & Roberts, G. J. (2002) Comparison of lysis filtration and an automated blood culture system (BACTEC) for detection, quantification, and identification of odontogenic bacteremia in children. *Journal of Clinical Microbiology* **40**, 3416–3420.

Lux, R., Miller, J. N., Park, N. H. & Shi, W. (2001) Motility and chemotaxis in tissue penetration of oral epithelial cell layers by Treponema denticola. *Infection and Immunity* **69**, 6276–6283.

Maeda, T., Maeda, H., Yamabe, K., Mineshiba, J., Tanimoto, I., Yamamoto, T., Naruishi, K., Kokeguchi, S. & Takashiba, S. (2010) Highly expressed genes in a rough-colony-forming phenotype of *Aggregatibacter actinomycetemcomitans*: implication of a mip-like gene for the invasion of host tissue. *FEMS Immunology and Medical Microbiology* **58**, 226–236.

Maekawa, T., Takahashi, N., Tabeta, K., Aoki, Y., Miyashita, H., Miyauchi, S., Miyazawa, H., Nakajima, T. & Yamazaki, K. (2011) Chronic oral infection with *Porphyromonas gingivalis* accelerates atheroma formation by shifting the lipid profile. *PLoS ONE* **6**, e20240.

Mahendra, J., Mahendra, L., Kurian, V. M., Jaishankar, K. & Mythilli, R. (2010) 16S rRNA-based detection of oral pathogens in coronary atherosclerotic plaque. *Indian Journal of Dental Research* **21**, 248–252.

Marcelino, S. L., Gaetti-Jardim, E. Jr, Nakano, V., Canonico, L. A., Nunes, F. D., Lotufo, R. F., Pustiglioni, F. E., Romito, G. A. & Avila-Campos, M. J. (2010) Presence of periodontopathic bacteria in coronary arteries from patients with chronic periodontitis. *Anaerobe* **16**, 629–632.

Marques da Silva, R., Caugant, D. A., Lingaas, P. S., Geiran, O., Tronstad, L. & Olsen, I. (2005) Detection of *Actinobacillus actinomycetemcomitans* but not bacteria of the red complex in aortic aneurysms by multiplex polymerase chain reaction. *Journal of Periodontology* **76**, 590–594.

Marques da Silva, R., Lingaas, P. S., Geiran, O., Tronstad, L. & Olsen, I. (2003) Multiple bacteria in aortic aneurysms. *Journal of Vascular Surgery* **38**, 1384–1389.

Martinet, W. & De Meyer, G. R. (2008) Autophagy in atherosclerosis. *Current Atherosclerosis Reports* **10**, 216–223.

Mastragelopulos, N., Haraszthy, V. I., Zambon, J. J. & Zafiropoulos, G. G. (2002) Detection of periodontal pathogenic microorganisms in atheromatous plaque. Preliminary results. *Der Chirurg; Zeitschrift fur alle Gebiete der operativen Medizen* **73**, 585–591.

Messini, M., Skourti, I., Markopulos, E., Koutsia-Carouzou, C., Kyriakopoulou, E., Kostaki, S., Lambraki, D. & Georgopoulos, A. (1999) Bacteremia after dental treatment in mentally handicapped people. *Journal of Clinical Periodontology* **26**, 469–473.

Meyer, D. H. & Fives-Taylor, P. M. (1997) The role of Actinobacillus actinomycetemcomitans in the pathogenesis of periodontal disease. *Trends in Microbiology* **5**, 224–228.

Meyer, D. H., Lippmann, J. E. & Fives-Taylor, P. M. (1996) Invasion of epithelial cells by *Actinobacillus actinomycetemcomitans*: a dynamic, multistep process. *Infection and Immunity* **64**, 2988–2997.

Meyer, D. H., Sreenivasan, P. K. & Fives-Taylor, P. M. (1991) Evidence for invasion of a human oral cell line by *Actinobacillus actinomycetemcomitans*. *Infection and Immunity* **59**, 2719–2726.

Moffatt, C. E., Inaba, H., Hirano, T. & Lamont, R. J. (2012) *Porphyromonas gingivalis* SerB-mediated dephosphorylation of host cell cofilin modulates invasion efficiency. *Cellular Microbiology* **14** (4), 577–588.

© 2013 European Federation of Periodontology and American Academy of Periodontology

Molmeret, M., Alli, O. A., Zink, S., Flieger, A., Cianciotto, N. P. & Kwaik, Y. A. (2002) icmT is essential for pore formation-mediated egress of *Legionella pneumophila* from mammalian and protozoan cells. *Infection and Immunity* **70**, 69–78.

Murphy, A. M., Daly, C. G., Mitchell, D. H., Stewart, D. & Curtis, B. H. (2006) Chewing fails to induce oral bacteraemia in patients with periodontal disease. *Journal of Clinical Periodontology* **33**, 730–736.

Nakano, K., Hokamura, K., Taniguchi, N., Wada, K., Kudo, C., Nomura, R., Kojima, A., Naka, S., Muranaka, Y., Thura, M., Nakajima, A., Masuda, K., Nakagawa, I., Speziale, P., Shimada, N., Amano, A., Kamisaki, Y., Tanaka, T., Umemura, K. & Ooshima, T. (2011) The collagen-binding protein of *Streptococcus mutans* is involved in haemorrhagic stroke. *Nature Communications* **2**, 485.

Nakano, K., Inaba, H., Nomura, R., Nemoto, H., Takeuchi, H., Yoshioka, H., Toda, K., Taniguchi, K., Amano, A. & Ooshima, T. (2008) Distribution of *Porphyromonas gingivalis fim*A genotypes in cardiovascular specimens from Japanese patients. *Oral Microbiology and Immunology* **23**, 170–172.

Nakano, K., Nemoto, H., Nomura, R., Homma, H., Yoshioka, H., Shudo, Y., Hata, H., Toda, K., Taniguchi, K., Amano, A. & Ooshima, T. (2007) Serotype distribution of *Streptococcus mutans* a pathogen of dental caries in cardiovascular specimens from Japanese patients. *Journal of Medical Microbiology* **56**, 551–556.

Nakano, K., Nemoto, H., Nomura, R., Inaba, H., Yoshioka, H., Taniguchi, K., Amano, A. & Ooshima, T. (2009) Detection of oral bacteria in cardiovascular specimens. *Oral Microbiology and Immunology* **24**, 64–68.

Nobbs, A. H., Lamont, R. J. & Jenkinson, H. F. (2009) *Streptococcus* adherence and colonization. *Microbiology and Molecular Biology Reviews* **73**, 407–450.

Noiri, Y., Ozaki, K., Nakae, H., Matsuo, T. & Ebisu, S. (1997) An immunohistochemical study on the localization of *Porphyromonas gingivalis, Campylobacter rectus* and *Actinomyces viscosus* in human periodontal pockets. *Journal of Periodontal Research* **32**, 598–607.

Ohki, T., Itabashi, Y., Kohno, T., Yoshizawa, A., Nishikubo, S., Watanabe, S., Yamane, G. & Ishihara, K. (2012) Detection of periodontal bacteria in thrombi of patients with acute myocardial infarction by polymerase chain reaction. *American Heart Journal* **163**, 164–167.

Okuda, K., Ishihara, K., Nakagawa, T., Hirayama, A. & Inayama, Y. (2001) Detection of *Treponema denticola* in atherosclerotic lesions. *Journal of Clinical Microbiology* **39**, 1114–1117.

Olczak, T., Maszczak-Seneczko, D., Smalley, J. W. & Olczak, M. (2012) Gallium(III), cobalt (III) and copper(II) protoporphyrin IX exhibit antimicrobial activity against *Porphyromonas gingivalis* by reducing planktonic and biofilm growth and invasion of host epithelial cells. *Archives of Microbiology* **194**, 719–724.

Oscarsson, J., Karched, M., Thay, B., Chen, C. & Asikainen, S. (2008) Proinflammatory effect in whole blood by free soluble bacterial components released from planktonic and biofilm cells. *BMC Microbiology* **8**, 206.

Ott, S. J., El Mokhtari, N. E., Musfeldt, M., Hellmig, S., Freitag, S., Rehman, A., Kuhbacher, T., Nikolaus, S., Namsolleck, P., Blaut, M., Hampe, J., Sahly, H., Reinecke, A., Haake, N., Gunther, R., Kruger, D., Lins, M., Herrmann, G., Folsch, U. R., Simon, R. & Schreiber, S. (2006) Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. *Circulation* **113**, 929–937.

Padilla, C., Lobos, O., Hubert, E., Gonzalez, C., Matus, S., Pereira, M., Hasbun, S. & Descouvieres, C. (2006) Periodontal pathogens in atheromatous plaques isolated from patients with chronic periodontitis. *Journal of Periodontal Research* **41**, 350–353.

Pan, N. J., Brady, M. J., Leong, J. M. & Goguen, J. D. (2009) Targeting type III secretion in *Yersinia pestis*. *Antimicrobial Agents and Chemotherapy* **53**, 385–392.

Perez-Chaparro, P. J., Gracieux, P., Lafaurie, G. I., Donnio, P. Y. & Bonnaure-Mallet, M. (2008) Genotypic characterization of *Porphyromonas gingivalis* isolated from subgingival plaque and blood sample in positive bacteremia subjects with periodontitis. *Journal of Clinical Periodontology* **35**, 748–753.

Peters, S. R., Valdez, M., Riviere, G. & Thomas, D. D. (1999) Adherence to and penetration through endothelial cells by oral treponemes. *Oral Microbiologyogy and Immunology* **14**, 379–383.

Pineiro, A., Tomas, I., Blanco, J., Alvarez, M., Seoane, J. & Diz, P. (2010) Bacteraemia following dental implants' placement. *Clinical Oral Implants Research* **21**, 913–918.

Pirro, M., Bagaglia, F., Paoletti, L., Razzi, R. & Mannarino, M. R. (2008) Hypercholesterolemia-associated endothelial progenitor cell dysfunction. *Therapeutic Advances in Cardiovascular Disease* **2**, 329–339.

Pober, J. S., Min, W. & Bradley, J. R. (2009) Mechanisms of endothelial dysfunction, injury, and death. *Annual Reviews Pathology: Mechanisms of Disease* **4**, 71–95.

Pollreisz, A., Huang, Y., Roth, G. A., Cheng, B., Kebschull, M., Papapanou, P. N., Schmidt, A. M. & Lalla, E. (2010) Enhanced monocyte migration and pro-inflammatory cytokine production by *Porphyromonas gingivalis* infection. *Journal of Periodontal Research* **45**, 239–245.

Popovic, M., Smiljanic, K., Dobutovic, B., Syrovets, T., Simmet, T. & Isenovic, E. R. (2012) Human cytomegalovirus infection and atherothrombosis. *Journal of Thrombosis and Thrombolysis* **33**, 160–172.

Prochnau, D., Lehmann, M., Straube, E., Figulla, H. R. & Rodel, J. (2011) Human cytomegalovirus induces MMP-1 and MMP-3 expression in aortic smooth muscle cells. *Acta Microbiologica et Immunologica Hungarica* **58**, 303–317.

Pucar, A., Milasin, J., Lekovic, V., Vukadinovic, M., Ristic, M., Putnik, S. & Kenney, E. B. (2007) Correlation between atherosclerosis and periodontal putative pathogenic bacterial infections in coronary and internal mammary arteries. *Journal of Periodontology* **78**, 677–682.

Rafferty, B., Jönsson, D., Kalachikov, S., Demmer, R. T., Nowygrod, R., Elkind, M. S., Bush, H. Jr & Kozarov, E. (2011) Impact of monocytic cells on recovery of uncultivable bacteria from atherosclerotic lesions. *Journal of Internal Medicine* **270**, 273–280.

Rajasuo, A., Perkki, K., Nyfors, S., Jousimies-Somer, H. & Meurman, J. H. (2004) Bacteremia following surgical dental extraction with an emphasis on anaerobic strains. *Journal of Dental Research* **83**, 170–174.

Raman, K., Chong, M., Akhtar-Danesh, G. G., D'Mello, M., Hasso, R., Ross, S., Xu, F. & Pare, G.(2013) Genetic markers of inflammation and their role in cardiovascular disease. *Canadian Journal of Cardiology* **29**, 67–74.

Razani, B., Feng, C., Coleman, T., Emanuel, R., Wen, H., Hwang, S., Ting, J. P., Virgin, H. W., Kastan, M. B. & Semenkovich, C. F. (2012) Autophagy links inflammasomes to atherosclerotic progression. *Cell Metabolism* **15**, 534–544.

Ridker, P. M. (2002) On evolutionary biology, inflammation, infection, and the causes of atherosclerosis. *Circulation* **105**, 2–4.

Rodrigues, P. H. & Progulske-Fox, A. (2005) Gene expression profile analysis of *Porphyromonas gingivalis* during invasion of human coronary artery endothelial cells. *Infection and Immunity* **73**, 6169–6173.

Rodrigues, P. H., Reyes, L., Chadda, A. S., Bélanger, M., Wallet, S. M., Akin, D., Dunn, W. Jr & Progulske-Fox, A. (2012) *Porphyromonas gingivalis* strain specific interactions with human coronary artery endothelial cells: a comparative study. *PLoS ONE* **7** (12), e52606.

Romano, F., Barbui, A. & Aimetti, M. (2007) Periodontal pathogens in periodontal pockets and in carotid atheromatous plaques. *Minerva Stomatologica* **56**, 169–179.

Roth, G. A., Ankersmit, H. J., Brown, V. B., Papapanou, P. N., Schmidt, A. M. & Lalla, E. (2007a) *Porphyromonas gingivalis* infection and cell death in human aortic endothelial cells. *FEMS Microbiology Letters* **272**, 106–113.

Roth, G. A., Moser, B., Huang, S. J., Brandt, J. S., Huang, Y., Papapanou, P. N., Schmidt, A. M. & Lalla, E. (2006) Infection with a periodontal pathogen induces procoagulant effects in human aortic endothelial cells. *Journal of Thrombosis and Haemostasis* **4**, 2256–2261.

Roth, G. A., Moser, B., Roth-Walter, F., Giacona, M. B., Harja, E., Papapanou, P. N., Schmidt, A. M. & Lalla, E. (2007b) Infection with a periodontal pathogen increases mononuclear cell adhesion to human aortic endothelial cells. *Atherosclerosis* **190**, 271–281.

Ross, R. (1999) Atherosclerosis – an inflammatory disease. *The New England Journal of Medicine* **340**, 115–126.

Rudney, J. D., Chen, R. & Sedgewick, G. J. (2001) Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. *Infection and Immunity* **69**, 2700–2707.

Rudney, J. D., Chen, R. & Sedgewick, G. J. (2005) *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*, and *Tannerella forsythensis* are components of a polymicrobial intracellular flora within human buccal cells. *Journal of Dental Research* **84**, 59–63.

Sabet, M., Lee, S. W., Nauman, R. K., Sims, T. & Um, H. S. (2003) The surface (S-) layer is a virulence factor of *Bacteroides forsythus*. *Microbiology* **149**, 3617–3627.

Saglie, F. R., Smith, C. T., Newman, M. G., Carranza, F. A. Jr, Pertuiset, J. H., Cheng, L., Auil, E. & Nisengard, R. J. (1986) The presence of bacteria in the oral epithelium in periodontal disease. II. Immunohistochemical identification of bacteria. *Journal of Periodontology* **57**, 492–500.

Saito, A., Inagaki, S., Kimizuka, R., Okuda, K., Hosaka, Y., Nakagawa, T. & Ishihara, K. (2008) *Fusobacterium nucleatum* enhances invasion of human gingival epithelial and aortic endothelial cells by *Porphyromonas gingivalis*. *FEMS Immunology & Medical Microbiology* **54**, 349–355.

Sakakibara, J., Nagano, K., Murakami, Y., Higuchi, N., Nakamura, H., Shimozato, K. & Yoshimura, F. (2007) Loss of adherence ability to human gingival epithelial cells in S-layer protein-deficient mutants of *Tannerella forsythensis*. *Microbiology* **153**, 866–876.

© 2013 European Federation of Periodontology and American Academy of Periodontology

Schenkein, H. A., Barbour, S. E., Berry, C. R., Kipps, B. & Tew, J. G. (2000) Invasion of human vascular endothelial cells by *Actinobacillus actinomycetemcomitans* via the receptor for platelet-activating factor. *Infection and Immunity* **68**, 5416–5419.
Scherz-Shouval, R. & Elazar, Z. (2007) ROS, mitochondria and the regulation of autophagy. *Trends in Cell Biology* **17**, 422–427.
Schrijvers, D. M., De Meyer, G. R. & Martinet, W. (2011) Autophagy in atherosclerosis: a potential drug target for plaque stabilization. *Arteriosclerosis, Thrombosis, and Vascular Biology* **31**, 2787–2791.
Shin, D. M., Yuk, J. M., Lee, H. M., Lee, S. H., Son, J. W., Harding, C. V., Kim, J. M., Modlin, R. L. & Jo, E. K. (2010) Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signalling. *Cell Microbiology* **12**, 1648–1665.
Silver, J. G., Martin, A. W. & McBride, B. C. (1977) Experimental transient bacteraemias in human subjects with varying degrees of plaque accumulation and gingival inflammation. *Journal of Clinical Periodontology* **4**, 92–99.
Silver, J. G., Martin, A. W. & McBride, B. C. (1979) Experimental transient bacteraemias in human subjects with clinically healthy gingivae. *Journal of Clinical Periodontology* **6**, 33–36.
Song, H., Belanger, M., Whitlock, J., Kozarov, E. & Progulske-Fox, A. (2005) Hemagglutinin B is involved in the adherence of *Porphyromonas gingivalis* to human coronary artery endothelial cells. *Infection and Immunity* **73**, 7267–7273.
Sreenivasan, P. K., Meyer, D. H. & Fives-Taylor, P. M. (1993) Requirements for invasion of epithelial cells by Actinobacillus actinomycetemcomitans. *Infection and Immunity* **61**, 1239–1245.
Stelzel, M., Conrads, G., Pankuweit, S., Maisch, B., Vogt, S., Moosdorf, R. & Flores-de-Jacoby, L. (2002) Detection of *Porphyromonas gingivalis* DNA in aortic tissue by PCR. *Journal of Periodontology* **73**, 868–870.
Suwannakul, S., Stafford, G. P., Whawell, S. A. & Douglas, C. W. (2010) Identification of bistable populations of *Porphyromonas gingivalis* that differ in epithelial cell invasion. *Microbiology* **156**. *Pt* **10**, 3052–3064.
Takahashi, Y., Davey, M., Yumoto, H., Gibson, F. C. 3rd & Genco, C. A. (2006) Fimbria-dependent activation of pro-inflammatory molecules in *Porphyromonas gingivalis* infected human aortic endothelial cells. *Cellular Microbiology* **8**, 738–757.
Takeuchi, H., Furuta, N., Morisaki, I. & Amano, A. (2011) Exit of intracellular *Porphyromonas gingivalis* from gingival epithelial cells is mediated by endocytic recycling pathway. *Cellular Microbiology* **13**, 677–691.
Taylor-Robinson, D., Aduse-Opoku, J., Sayed, P., Slaney, J. M., Thomas, B. J. & Curtis, M. A. (2002) Oro-dental bacteria in various atherosclerotic arteries. *European Journal of Clinical Microbiology & Infectious Diseases* **21**, 755–757.
Tomas, I., Alvarez, M., Limeres, J., Potel, C., Medina, J. & Diz, P. (2007a) Prevalence, duration and aetiology of bacteraemia following dental extractions. *Oral Diseases* **13**, 56–62.
Tomas, I., Alvarez, M., Limeres, J., Tomas, M., Medina, J., Otero, J. L. & Diz, P. (2007b) Effect of a chlorhexidine mouthwash on the risk of postextraction bacteremia. *Infection Control and Hospital Epidemiology* **28**, 577–582.
Tomas, I., Diz, P., Tobias, A., Scully, C. & Donos, N. (2012) Periodontal health status and bacteraemia from daily oral activities: systematic review/meta-analysis. *Journal of Clinical Periodontology* **39**, 213–228.
Tomas, I., Pereira, F., Llucian, R., Poveda, R., Diz, P. & Bagan, J. V. (2008) Prevalence of bacteraemia following third molar surgery. *Oral Diseases* **14**, 89–94.
Tribble, G. D. & Lamont, R. J. (2010) Bacterial invasion of epithelial cells and spreading in periodontal tissue. *Periodontology 2000* **52**, 68–83.
Vita, J. A. & Loscalzo, J. (2002) Shouldering the risk factor burden: infection, atherosclerosis, and the vascular endothelium. *Circulation* **106**, 164–166.
Wan, M. & Hajishengallis, G. (2008) Lipid raft-dependent uptake, signalling and intracellular fate of *Porphyromonas gingivalis* in mouse macrophages. *Cellular Microbiology* **10**, 2029–2042.
Wang, M., Liang, S., Hosur, K. B., Domon, H., Yoshimura, F., Amano, A. & Hajishengallis, G. (2009) Differential virulence and innate immune interactions of Type I and II fimbrial genotypes of *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **24**, 478–484.
Winkler, J. R., Matarese, V., Hoover, C. I., Kramer, R. H. & Murray, P. A. (1988) An in vitro model to study bacterial invasion of periodontal tissues. *Journal of Periodontology* **59**, 40–45.
Woollard, K. J. & Geissmann, F. (2010) Monocytes in atherosclerosis: subsets and functions. *Nature Cardiology in Review* **7**, 77–86.
Wu, H., Lippmann, J. E., Oza, J. P., Zeng, M., Fives-Taylor, P. & Reich, N. O. (2006) Inactivation of DNA adenine methyltransferase alters virulence factors in *Actinobacillus actinomycetemcomitans*. *Oral Microbiology and Immunology* **21**, 238–244.
Xie, Y., You, S. J., Zhang, Y. L., Han, Q., Cao, Y. J., Xu, X. S., Yang, Y. P., Li, J. & Liu, C. F. (2011) Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells. *Molecular Medicine Reports* **4**, 459–464.
Yamatake, K., Maeda, M., Kadowaki, T., Takii, R., Tsukuba, T., Ueno, T., Kominami, E., Yokota, S. & Yamamoto, K. (2007) Role for gingipains in *Porphyromonas gingivalis* traffic to phagolysosomes and survival in human aortic endothelial cells. *Infection and Immunity* **75**, 2090–2100.
Yumoto, H., Chou, H. H., Takahashi, Y., Davey, M. & Gibson, F. C., 3rd, & Genco, C. A. (2005) Sensitization of human aortic endothelial cells to lipopolysaccharide via regulation of Toll-like receptor 4 by bacterial fimbria-dependent invasion. *Infection and Immunity* **73**, 8050–8059.
Zaremba, M., Gorska, R., Suwalski, P. & Kowalski, J. (2007) Evaluation of the incidence of periodontitis-associated bacteria in the atherosclerotic plaque of coronary blood vessels. *Journal of Periodontology* **78**, 322–327.
Zeituni, A. E., Jotwani, R., Carrion, J. & Cutler, C. W. (2009) Targeting of DC-SIGN on human dendritic cells by minor fimbriated *Porphyromonas gingivalis* strains elicits a distinct effector T cell response. *Journal of Immunology* **183**, 5694–5704.
Zeituni, A. E., McCaig, W., Scisci, E., Thanassi, D. G. & Cutler, C. W. (2010) The native 67-kilodalton minor fimbria of *Porphyromonas gingivalis* is a novel glycoprotein with DC-SIGN-targeting motifs. *Journal of Bacteriology* **192**, 4103–4110.
Zhang, W., Ju, J., Rigney, T. & Tribble, G. D. (2011) Fimbriae of *Porphyromonas gingivalis* are important for initial invasion of osteoblasts, but not for inhibition of their differentiation and mineralization. *Journal of Periodontal Research* **82**, 909–916.
Zhang, Y. L., Cao, Y. J., You, S. J., Li, R. X., Liu, H. H. & Liu, C. F. (2010a) Protective effects of autophagy against oxidized LDL-induced injury in endothelial cells. *Zhonghua Yi Xue Za Zhi* **90**, 2792–2796.
Zhang, Y. M., Zhong, L. J., Liang, P., Liu, H., Mu, L. T. & Ai, S. K. (2008) Detection of periodontal pathogenic bacteria DNA in coronary atheromatous plaques from patients underwent coronary artery bypass graft. *Zhonghua Xin Xue Guan Bing Za Zhi* **36**, 215–218.

Address:
Ann Progulske-Fox, Department of Oral Biology, Center for Molecular Microbiology, PO Box 100424 JHMHSC, University of Florida, Gainesville, FL 32610-0424, USA
E-mail: apfox@dental.ufl.edu

Clinical Relevance

Scientific rationale for the study: A link between cardiovascular diseases and periodontal diseases has been observed in both epidemiological studies and in animal models of disease. This review was undertaken to evaluate the evidence that periodontal pathogen dissemination contributes to the biological plausibility of the link.

Principal findings: The evaluation of the seven "proofs" of evidence proposed in the review support the possible role of periodontal pathogens in the association between periodontal and cardiovascular diseases.

Practical implications: These findings establish that additional experimentation is necessary to determine if prevention and treatment of periodontal diseases would disrupt the link with cardiovascular diseases.

© 2013 European Federation of Periodontology and American Academy of Periodontology