TITLE

Autophagy in periodontal disease: Evidence from a literature review.

AUTHORS: Alejandro I. Lorenzo-Pouso[1]; Pablo Castelo-Baz, [2]; Mario Pérez-Sayáns[1]; Jason Lim[3]; Yago Leira[3,4]

[1]Oral Medicine, Oral Surgery and Implantology Unit, Faculty of Medicine and Odontology, University of Santiago de Compostela, GI-1319 Research Group, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain.
[2]Endodontics Unit, Faculty of Medicine and Odontology, University of Santiago de Compostela, Santiago de Compostela, Spain.
[3]Periodontology Unit, UCL Eastman Dental Institute and Hospital, University College London, London, UK.
[4]Periodontology Unit, Faculty of Medicine and Odontology, University of Santiago de Compostela, Medical-Surgical Dentistry (OMEQUI) Research Group, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain.

Address for correspondence

Dr. Alejandro I. Lorenzo-Pouso
Oral Medicine, Oral Surgery and Implantology Unit– Faculty of Medicine and Odontology
University of Santiago de Compostela
Rúa Entrerríos S/N
15782 - Santiago de Compostela (Spain)
E-mail: alexlopo@hotmail.com
Phone: +34 636676784

RUNNING TITLE: Autophagy in periodontal disease

ABSTRACT

Objective: To summarize evidence and data in relation to the implications of autophagy in periodontal disease (PD) and describe potential nutraceuticals or pharmaceuticals able to modulate this subtype of cell death.

Design: Literature searches of different electronic databases (Medline via PubMed, SCOPUS, Web of Science, and EMBASE) using appropriate keywords (e.g. periodontal disease, periodontitis, alveolar bone loss, periodontal infection, tooth loss, autophagy, programmed cell death, type 2 cell death) were performed. Then, a comprehensive literature review of the current understanding of this link was elaborated.

Results: Autophagy plays a pivotal role in PD and it seems that its regulation may be an interesting avenue for future periodontal research according to several *in vivo* and *in vitro* reports.

Conclusion: Nowadays research has ascertained the role of autophagy in PD, specially its role in the host defence against periodontal disease drivers. A bulk of research has recognized several pharmaceuticals and nutraceuticals that can potentially modulate this kind of cell death and serve as useful therapies. However, further research is warranted in order to reach a clinical translation, which could be of help in the discovery of novel host modulation therapies for PD.

KEY WORDS: autophagy; periodontal disease; disease prevention; molecular targets; redox regulation; cell fate.

Conflict of interest and source of funding

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Autophagy in periodontal disease: Evidence from a literature review.

1. Introduction

Autophagy is an evolutionarily conserved lysosome-dependent catabolic process (Klionsky and Emr, 2000). This degradation process exerts a crucial influence on multiple physiological processes such as cellular homeostasis, aging, development, host-pathogen interactions, differentiation, and cell death and survival (Levine and Kroemer, 2008). In the last two decades, autophagy-related pathways and autophagy-related genes (ATGs) have been extensively studied in yeast and human as model organism (Takeshige et al., 1992, Mizushima et al., 2010).

Under basal conditions, autophagy occurs in most eukaryotic cells allowing the degradation and recycling of surplus and damaged cellular components. In disease, dysfunction of the autophagy process has been linked to several pathological conditions (Giampieri et al., 2018). This connection stems from autophagy role in quality control of the proteome, organelles and cellular energetic balance. Autophagy is not only activated in response to cellular damage (i.e., oxidative stress, endoplasmic reticulum stress etc.), more challenging insult-induced adaptive responses can trigger autophagy such as nutrient and growth factor withdrawal, infection, hypoxia, or heat (Galluzzi et al., 2017). This mechanism also plays a pivotal role during cellular starvation producing an increase in ATP levels by protein breakdown (Zhang et al., 2018). Some dying eukaryotic cells exhibit highly enhanced autophagic activity. This kind of cell lyses has been named as Type 2 Cell Death (T2CD) due to its distinctive biological signature in relation to Type 1 Cell Death (T1CD) or apoptosis (Galluzzi et al., 2018).

The search of pharmacological agents and nutrition interventions which have may have potential as autophagy regulators represents an interesting avenue for future research. In this sense, recent reports have shown novel autophagy-based therapeutics for a raising number of diseases (Rubinsztein et al., 2012). Despite these trends, to date there is no single drugs approved by the US Food and Drug Administration (FDA) exclusively as an autophagy regulator (Levine et al., 2015). Unfortunately, the knowledge available regarding autophagy-related pathways remains limited and emerging frontiers

jeopardize its interference as a therapeutic target (Giampieri et al., 2018). At a genetic level, several genetic aberrations in ATGs have been linked to the susceptibility of an increasing number of diseases (van Beek et al., 2018).

There are 3 main types of autophagy: macroautophagy (MA), microautophagy (mA) and chaperone-mediated autophagy (CMA). The different forms of autophagy mainly differ in the mode of cargo delivery to the lysosome. The best characterised is MA, which in turns is frequently identified simply as ¨autophagy¨. MA (hereafter simply referred to as autophagy unless specified) can be divided into different phases: induction, elongation, maturation, transport to lysosomes, and degradation (Mizushima, 2007). A concise update on its mechanisms is shown on Fig. 1. mA is a form of cargo degradation in which targeted cytoplasmatic materials are directly engulfed by the vacuole constituting multivesicular bodies (MVBs), subsequently cargo is cleaved by enzymes (Sahu et al., 2011). CMA does not require the formation of MVBs; proteins are directly internalized in lysosomal lumen. This kind of autophagy is highly selective; it just captures proteins with the (Lys-Phe-Glu-Arg-Gln) KFERQ motif (Cuervo and Wong, 2014).

MA is the focus of the present review. Several pathologies has been linked to this biological process since in 1999 Liang et al. discovered its beclin 1-dependant induction/inhibition by means of gene-transfer techniques in MCF-7 cells (Liang et al., 1999). In this vein, a significant and increasing number of diseases have been linked to autophagy (eg. cancer, autoimmune diseases, metabolic disorders, neurodegeneration, or pathogen infection) (Levine et al., 2015). The role of autophagy in disease is context dependent, this fact has been named as the ¨Autophagy paradox¨. In this line, defective autophagy promotes the accumulation of proteins and potentially hazardous intracellular structures providing a protective environment for several hallmarks of diseases. On the other line, functional autophagy exerts death promoting roles to protect cells from insults. However, the underlying mechanism that regulates the autophagy paradox remains unclear (Mizushima et al., 2010). In 2004, three research articles provided the first evidences regarding autophagy role in bacterial infection (Gutierrez et al., 2004, Ogawa et al., 2005, Nakagawa et al., 2004).

Periodontal disease (PD) is a chronic oral disease produced by a bacterial insult that triggers an altered immune response (Page and Kornman, 1997). A recent meta-

regression analysis showed that severe PD affected the 11.2% of the global population between 1990 and 2010 (Kassebaum et al., 2014). PD has been strongly linked to the likelihood of suffering from different groups of human diseases such as cardiovascular diseases (CVDs), neuroinflammatory diseases, or diabetes (Leira et al., 2017a, Leira et al., 2017b, D'Aiuto et al., 2017). In turn, these outcomes have been heavily linked to autophagy dependant mechanisms (Hara et al., 2006, Feng et al., 2017). A possible hypothesis for this epidemiological relationship may be the effect of systemic inflammation on the danger-associated molecular patterns (DAMPs) and pathogens ability to build pathogen-associated molecular patterns (PAMPs). Thus, recent research has focused on elucidate the link between autophagy and PD.

In the oral microenvironment, inflammation is often activated by a single bacteria knows as *P. gingivalis*, which triggers the Toll-Like Receptor (TLR) pathway (Bostanci and Belibasakis, 2012). *P. gingivalis*-related survival can be achieved by the subversion of the host autophagic pathway, meaning a relevant innate immune interaction (Liu et al., 2018). Other molecular bases for pathogenesis of PD have proven relation with autophagy such as reactive oxygen species (ROS), neutrophil extracellular traps, and chaperones (Hirschfeld et al., 2017, Goulhen et al., 2003). Recently several therapeutic agents that can exert autophagy modulation *in vivo* have been identified for several chronic diseases (Giampieri et al., 2018). In the case of PD, a few agents have been identified as autophagy modulators in several cell lines of different histotypes and animal models, establishing interesting therapeutic targets (Couve et al., 2013). Thus, the autophagy pathway interference can mean a non-mainstream and poorly explored approach against PD.

Although three reviews relating to the association between PD and autophagy have been carried out, none of them has focused on the potential of autophagy interference as a therapy for PD, the autophagy-dependent links between systemic diseases and PD, or its relation with other relevant biological pathways implicated on the aetiology of PD (Tan et al., 2017, Liu et al., 2017, Jiang et al., 2019). Moreover, these reviews dealt with the linkage between only in a section of the full review (Tan et al., 2017), treated exclusively a subtype of autophagy (i.e., pexophagy) (Liu et al., 2017), or did not provide critical remarks and recommendations on future research in this area (Jiang et al., 2019).

This review aims to explore existing literature regarding autophagy on PD, offer new perspectives in relation to the discovery of novel treatment possibilities, and in the search of mechanisms to alleviate periodontal tissues immune senescence.

2. Material and methods

The literature search was performed independently by 2 reviewers (AILP and YL). An electronic search was performed on the following databases: Medline via PubMed, SCOPUS, Web of Science, and EMBASE. Studies published in the last ten years, before 31, November 2018, with full text available were considered for inclusion. For the MEDLINE/PubMed the next keywords as MESH terms or free words were used: (periodontal disease OR periodontitis OR alveolar bone loss OR periodontal infection OR tooth loss) AND (autophagy OR programmed cell death OR type 2 cell death). Additional publications screened from citations of selected papers, recovered from the excluded group or resulting from manual searches in the most relevant periodontics and autophagy journals, were also included. Paper selection and data extraction were performed independently by aforementioned researchers. Any disagreement was resolved by consulting a third investigator (MPS).

Research articles describing: 1) Implications of autophagy in periodontal-related disease drivers, 2) autophagic-dependant links between PD and systemic diseases, 3) the relationship between autophagy in PD and other mechanisms, and 4) the protective effects of pharmaceuticals and nutraceuticals *in vitro* and/or *in vivo* models of PD by regulating autophagy, 5) novel directions of autophagy research in PDs were selected for further evaluation. There were no exclusion criteria in what concerns the study design. Studies in other language than English, and articles abstracts were excluded. Then, evidences were presented in the form of a narrative review. In the case of autophagy interferers, particular attention has been paid to their molecular targets in *in vitro* and *in vivo* studies, as well as to the diverse clinical trials that have confirmed them to be potential therapeutic agents for PD.

3. Findings

3.1. Implications of autophagy in periodontal-related disease drivers

The implications of autophagy in PD are not fully understood, however, it seems that it may play several roles in protecting cells from apoptosis, increasing angiogenesis in periodontal tissues, promoting other types of cell death, and providing bacteria with a route to evade the host response (Tsuda et al., 2012, Tan et al., 2017).

Higher levels of autophagy activity have been found in patients with PD compared with subjects with a healthy periodontal status (Bullon et al., 2012, An et al., 2016). Bullon et al. found increased levels of mitochondrial ROS and autophagy gene expression (LC3) in peripheral blood mononuclear cells of periodontal patients compared to periodontally healthy controls (Bullon et al., 2012). In addition, an *in vitro* investigation found that human gingival fibroblasts treated with lipopolysaccharide (LPS) from *P. gingivalis* had an increased expression of autophagy-related genes Atg-12 and LC3. Inhibition of autophagy with 3-MeA in LPS-treated gingival fibroblasts resulted in an increased rate of cell death by apoptosis, suggesting a protective role of autophagy (Bullon et al., 2012). An et al. found increased levels of LC3 gene expression in periodontal ligament (PDL) tissues from patients with PD compared to non-PD individuals (An et al., 2016). According to previous results, activation of autophagy in PDL stem cells (PDLSCs) also protected these cells from apoptosis (An et al., 2016). The same research group found that PDLSCs exposed to inflammatory environments had increased levels of autophagy and that autophagy promoted angiogenesis (Wei et al., 2018). PDLSCs were isolated from healthy tissues and inflamed periodontal tissues. In addition, a subgroup of PDLSCs from the healthy tissues were subsequently exposed to inflammatory cytokines *in vitro* and it was demonstrated that the cells exposed to inflammatory environments expressed basic fibroblast growth factor (bFGF) and angiogenin (ANG), both markers of angiogenesis. This increase in angiogenesis was also observed when autophagy was induced by treatment of cells with rapamycin and cDNA-Beclin-1. Therefore, autophagy appears to promote angiogenesis in PD (Wei et al., 2018).

Butyrate is a short-chain fatty acid produced by anaerobic bacteria capable of causing direct damage to host cells due to its toxicity. An *in vitro* experiment found that treating gingival epithelial cells with butyrate induced cell death (Tsuda et al., 2010). Inhibition of apoptosis with a pan-caspase inhibitor Z-VAD-FMK did not completely suppress cell death and the presence of autophagic cell death was confirmed by the higher levels of LC3-I conversion to LC3-II in butyrate treated cells compared to control (Tsuda et al.,

2010). Therefore, the presence of butyrate induced cell death by both apoptosis and autophagy. Ebe et al. found increased levels of necrotic cells in gingival epithelium associated with higher levels of butyrate (Ebe et al., 2011). It has been shown that gingival epithelial cells exposed to butyrate underwent necrosis and had the capability of releasing of high-mobility group-box 1 protein (HMGB1). HMGB1 can be classed as a DAMP due to its action as a pro-inflammatory mediator when released from cells. It can be a signalling molecule for recruitment of polymorphonucleocytes (PMNs) as well as being able to bind to pro-inflammatory receptors such as receptor for advanced glycations end-products (RAGE), and TLRs 2 and 4. In PD, higher quantities of anaerobic bacteria in subgingival biofilm may therefore be associated with increased levels of butyrate, HMGB1 release, and autophagic cell death (Tsuda et al., 2012).

P. gingivalis is a periodontal pathogen with the ability to manipulate the processes of autophagy to promote its own survival and invasion into host tissues. Fluorescence *in situ* hybridization techniques have confirmed the invasion of gingival epithelium by periodontal bacteria strongly associated with PD including *P. gingivalis, T. forsythia, T. denticola,* and *A. actinomycetemcomitans* (Colombo et al., 2007). After invading host cells, *P. gingivalis* can be found in multiple locations including cell cytoplasm, endosomes, and autophagosomes (Amano et al., 2014). The contents of autophagosomes are usually destroyed by fusion with lysosomes, however, *P. gingivalis* can impair the formation of autolysosomes allowing it to survive and receive nutrients from the autophagosome. Approximately half of *P. gingivalis* in endosomes is transported to lysosomes for degradation (Takeuchi et al., 2011). A significant proportion of remaining bacteria are reported to escape the lytic process through a recycling pathway for exocytosis. Once exited from the initially infected cell, the bacteria are able to enter adjacent cells, thereby penetrating further into periodontal tissues. Furthermore, it was found that approximately 10% of exited bacteria re-entered into new cells *in vitro* (Takeuchi et al., 2011).

3.2 Autophagic-dependant links between periodontal disease and systemic diseases

Periodontitis is associated with a great number of systematic pathologies by several underlying pathophysiological mechanisms, such as bacteraemia, endotoxemia or the release of inflammatory mediators (Van Dyke and van Winkelhoff, 2013). Up to now, PD has been linked to 57 systemic conditions (Monsarrat et al., 2016). Three of them

were selected [i.e., Alzheimer's disease (AD), ischemic stroke, and type II diabetes (T2D)] and its described autophagy-related hallmarks and possible relationships with PD are discussed.

AD is the most common form of neurodegeneration among elderly people. The molecular hallmarks of AD are the accumulation of amyloid beta (Aβ) and the hyperphosphorylation of tau in the brain (Reitz et al., 2011). Clinical evidence has ascertained the link AD-PD, in this line a recent meta-analysis reported an OR 1.69, 95% (CI 1.21-2.35) (Leira et al., 2017a). On the basis of the contribution of this local assault to systemic inflammation it is plausible that PD may be a trigger for AD (Kamer et al., 2008). Positron emission tomography imaging has ascertained Aβ accumulation in AD-susceptible brain areas in PD-affected patients vs. controls (Kamer et al., 2015) as the analysis of Aβ peptide concentration in serum confirmed a differential expression in periodontally compromised individuals (Gil-Montoya et al., 2017). Also *in vitro* reports have given insight into the ability of periodontopathogens-related LPS to create PAMPs and act upon these hallmarks (Pritchard et al., 2017). A great of deal of evidence has lately found in autophagy a possible therapeutic target against neurodegeneration. AD brains have shown an augmented accumulation of immature autophagic vacuoles that may impede the neuroprotective effects of autophagy via an immuno-electron microscopy study. (Nixon et al., 2005). Zheng et al. showed that Aβ production was suppressed 3-methyl adenine-dependent autophagy inhibition *in vitro* (Zheng et al., 2011). Aβ can be synthesized in the autophagosomes, which contain key mediators in its genesis (i.e., amyloid precursor protein and Presenilin-1) (Bustos et al., 2017). Also, tau post-translational modification can disrupt axoplasmic flow via autophagy, specifically through dynein-dynactin motors *in vitro* (Ikenaka et al., 2013).

Ischemic stroke implicates the deposit of a blood clot or fatty deposit block on a brain blood vessel. This subtype of CVD represents the 80% of all strokes (Warlow, 1998). A relationship between this CVD and PD has been reported in several epidemiological reports (quantified as RR 2.88 [1.53–5.41], according to a recent meta-analysis) (Leira et al., 2017b). These diseases can be linked specially by two mechanisms endothelial dysfunction, and invasion across the blood-brain barrier (BBB) (Leira et al., 2018, Pussinen et al., 2004, Pussinen et al., 2007). Autophagy modulates the differentiation and proliferation of endothelial progenitor cells (EPCs) and the equilibrium between

T2CD and T1CD in mature endothelial cells (ECs). In this line, ECs damage have been linked to redox-induced autophagy by several *in vitro* studies (Sachdev and Lotze, 2017). BBB disruption has been classically linked to the ability of matrix metalloproteinase to facilitate an increase in vascular permeability by degrading tight junction (TJ) proteins (Gonzalez-Mariscal et al., 2003). Nonetheless, histone deacetylase (HDAC) 9-mediated suppressed autophagy has been recently considered a relevant signal transduction pathway that may link cerebral injury to brain epigenetics (Shi et al., 2016).

T2D is a metabolic disorder whose main features are high blood sugar, insulin resistance, and a sub-optimal insulin concentration (Reaven et al., 1988). Successful PD management has proved effectiveness in the management of metabolic control, and systemic inflammation in T2D-affected patients (D'Aiuto et al., 2018). Hyperglycemia triggers a metabolic route (DAG-PKC-NADPH-oxidase) which generates ROS production; the secondary β-cell death to this route represents a relevant molecular explanation for diabetic complications (Stefano et al., 2016). PKC and mTORC1 interaction regulates apoptosis-autophagy equilibrium. In this line, PKC inhibition has proven an interesting avenue for autophagy-related T2D therapies (Das Evcimen and King, 2007).

A Plethora of plausible mechanisms behind the relationship between PD and systemic diseases is available, and autophagy seems to be one of them. Further research may contribute towards the development of novel host modulation therapies (MHTs) able to be acts as a two-edged sword.

3.3 The relationship between autophagy in periodontal disease and other mechanisms: Neutrophil Extracellular, and Reactive Oxygen Species and Chaperones

As in other bacterial assaults during PD, PMNs efflux is produced as a host response to pathogens. Specially, neutrophils are essential elements of host resistance to PD due to their potential to act upon the disruption of PAMPs (Genco, 1992). Nevertheless, the ability of some PD drivers to evade neutrophil microbicidal machinery and to produce a delayed apoptosis can turn this defensive cell activity into unwanted immune responses (Nussbaum and Shapira, 2011). To illustrate this point several quantitative and/or

qualitative alterations at the level of neutrophils secondary to genetic aberrations have been linked to PD aggressive forms (i.e., neutropenia, leukocyte adhesion deficiency, Chediak-Higashi syndrome, Papillon-Lefèvre syndrome, chronic granulomatous disease or Kostmann syndrome) (Hajishengallis and Hajishengallis, 2014, Carlsson et al., 2006).

NETs are extracellular chromatin structures that can trap and degrade microbes. This feature has helped in the identification of novel form cell death named NET cell death, or NETosis (Brinkmann et al., 2004). NET-associated host damage has been linked to the onset of several immune an infection-related conditions such as PD (White et al., 2016). NETs exert DNA backbone activities but also can release several active proteases to the ECM leading to bacterial lyses (Levy, 2004). On the other side, bacterial entrapment by NETs is highly variable according to the specific targeted bacteria, due to the variable capacity of each bacterium to release nucleases into the ECM (Papayannopoulos, 2014). Some bacterial virulence factors, such as *A. actinomycetemcomitans* leukotoxin, can induce NETosis. NETs can be stimulated by periodontopathogens and other microbes in anaerobic conditions (Lopes et al., 2018). These capacities are especially increased in periodontopathogens of the orange and red complexes according to Socransky classification (Socransky et al., 1998). The production of these web-like structures relies on the generation of ROS following assembly of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (White et al., 2016).

ROS are chemically reactive chemical species containing oxygen (Table 1). ROS can exert positive function under physiological conditions. However, increased ROS levels can trigger cellular microenvironment remodelling, which induces a phenomenon called oxidative stress (OS). This condition is necessarily accompanied by a reduction of the effectiveness of antioxidant systems such as endogenous enzymes like catalase, superoxide dismutase, and glutathione peroxidase, or exogenous factors such as micronutrients deficiency (i.e., polyphenols, vitamins, trace elements etc.) (Battino et al., 1999). Endogenous ROS major producer is mitochondria; nonetheless these radicals can also be generated by other systems such as NADPH enzyme activity, or oxidative protein folding at endoplasmic reticulum (Zhang et al., 2011). Mitochondrial oxidative stress production (mtROS) can damage periodontal ligament collagen fibres and ECM and trigger the production of several pro-inflammatory cytokines (D'Aiuto et al., 2010).

PNMs present in periodontal pockets create a microenvironment in relation to oxygen tension and pH (a medium of 1.8% and 6.92, respectively) capable of accommodating O_2^- (Waddington et al., 2000).

The ROS effects on periodontium have opened an interesting perspective in the treatment of this outcome by the use of exogenous antioxidants or modulators of endogenous antioxidants. In this line, limited evidence regarding the usefulness of compounds with antioxidant capacity as local adjuvant therapy or nutraceuticals for PD is available. Among them, Coenzyme Q_{10} and vitamin D are highlighted (Varela-Lopez et al., 2015, Varela-Lopez et al., 2018). Clinical trials applying classical non-surgical periodontal therapy have proven usefulness in the reduction of some OS-related biomarkers such as 8-hydroxydeoxiguanosine, total oxidant status, and total antioxidant status (da Silva et al., 2018). Recent advances in PD basic research have discovered a myriad of possible mechanism that can underlie these outcomes. Yoon et al. recently discovered that superoxide dismutase 2 (SO2) silencing increased production of NLRP3 inflammasome components, which elucidated the defensive role of SO2 in periodontal tissue probably via the regulation of NF-κB pathway *in vitro* (Yoon et al., 2018).

The cross-talk between ROS-NETs has been poorly explored. Recently, Chapple´s group hypothesized that bacterium-stimulated NET release may not arise via NADPH oxidase (Hirschfeld et al., 2017), and Hazeldine et al. demonstrated that reduced NET production was not linked to aging-related PD (Hazeldine et al., 2014).

ROS inhibition via antioxidants delays both the onset of autophagy and its related kinetics (Colotta et al., 1992). Redox imbalance can act on three substrates implicated in the autophagic pathway: 1) mTORC1, 2) beclin 1, and 3) Atg12-Atg5. Recently Atg5 aging-related defects have been linked to an impaired NET formation. In this sense, the interference at the level of autophagy-promoted NETs may be a promising as a therapeutic target for chronic PD due to its link with aging (Xu et al., 2017). Autophagy can be a contributor factor in the onset of NETosis, but superoxide is not *per se* required for the induction of PNMs autophagy *in vitro* (Fuchs et al., 2007). Discussed literature shows a complex interaction between ROS, autophagy and NET formation in neutrophils following infections.

Elevated ECM calcium can trigger the differentiation of human periodontal ligament stem cells via two mechanism calcium-sensing receptor (CaSR) and L-type voltage-dependent calcium channel. CaSR can activate NLRP3 inflammasome via autophagy in a process involving Hsp70 and LC3-II *in vitro* (Koori et al., 2014). Periodontal ligament cells treated with recombinant human inducible Hsp70 subjected to compressive forces induce an augmented expression of TNF-α and RANKL *in vitro* (Mitsuhashi et al., 2011). In this line, Hsp70 plays a pivotal role in periodontal physiology, and its control may be related to autophagy (Wolf et al., 2019).

3.4 Novel autophagy-dependent therapeutic targets for periodontal diseases

The main hallmarks of periodontal disease in which autophagy can interfere are: 1) the regulation of PD-related disease drivers, 2) the interference with secondary immunity and inflammation, and 3) the modulation of other kinds of cell death. Pharmacological autophagy inhibitors and inducers can be largely classified as early- or late-stage interferers. It is worth mentioning that some of these compounds can exert synergistic activities. In this line, targeting autophagy can mean the induction of citoprotective functions, but depending on the extent of autophagy, autophagy flux, and apoptosis competence these protective roles can flip to an enhancement in cytotoxicity (Tan et al., 2017). Unfortunately, given current limitations in measuring autophagy in patients, what constitutes a “normal” range of autophagy activity remains unknown (Levine et al., 2015).

Many autophagy regulators have been used in clinical medicine for years. In this vein, several FDA approved drugs have proven interference in the autophagic pathway, as some bioactive compounds and micronutrients have done. Nonetheless, several novel drugs are being tested in the search of therapeutic options by means of cell cultures and/or animal models and even some of them progressed to Phase I and II trials (Galluzzi et al., 2017). Table 1 summarizes some of these compounds and its checkpoints in the autophagic pathway.

Limited literature regarding the use of these compounds as a therapy for PDs is available, and most of this research is based on *in vitro* experiments. On the other hand, HMTs as a concept, in which drug therapies are used as adjuvant therapies, is still not a reality in clinical periodontology as the use of dietary supplement or diet interventions

is not. Until today, the only FDA recognized adjuvant therapy for this outcome is subantimicrobial dose doxycycline. Recent research has puts its focus on the benefits of novel local and systemic interventions as adjuncts to traditional scaling and root planning (SRP) (Ritchie et al., 2002, Moro et al., 2018). Below, a summary autophagic interferers that has proven effectiveness as HMTs are from *in vitro* experiments to *in vivo* and clinical studies

3.4.1 Natural bioactive compounds or derivatives

Functional food ingredients containing bioactive compounds introduced systemically or locally can exert significant biological effects and at the same time minimal side effects on the human body (Perez-Gregorio and Simal-Gandara, 2017). In this vein, nutrition is meaning a turning point in current periodontology (Dommisch et al., 2018). Chemical structures are shown in Fig. 2.

Maresin 1 (MaR1), a 12-lipoxygenase-derived metabolite of docosahexaenoic acid (DHA), can play a functional role in resolving inflammation, inducing autophagy and inhibiting apoptosis in human PDL cells via glycogen synthase kinase-3β/β-catenin pathway, according to an *in vitro* artificially induced inflammatory microenvironment (Du et al., 2018). Wang et al. also reported that *in vitro* cultured macrophages derived from monocytes isolated from peripheral blood of patients with localized aggressive PD have a lower concentration MaR1 vs. controls (Wang et al., 2015). Coincidently a recent systematic review of human trials has verified the relationship between low Omega−3 fatty acids (n-3) (i.e., eicosapentaenoic acid [EPA], DHA, and aracdonic acid [AA]) plasma concentrations and PD progression (Azzi et al., 2018).

Genistein is an isoflavone classically described as a potent antioxidant and antibacterial. This bioactive compound can regulate the IL-1β induced activation of MAPKs in PDL via G protein-coupled receptor 30 (Luo et al., 2012). Genistein has proven usefulness in the attenuation of some *Prevotella intermedia*-LPS induced inflammation products (i.e., IL-6 and nitrogen reactive species [NOS]) in an *in vitro* study of murine macrophages (Choi et al., 2016). Bhattarai et al. demonstrated the genistein *in vitro* ability to protect periodontium via autophagy induction using RAW 264.7 macrophages and human gingival fibroblasts (hGFs) (Bhattarai et al., 2017). Cinnamaldehyde, a naturally synthesized flavonoid by the shikimate pathway, inhibits pro-inflammatory cytokines

secretion via NF-κB downregulation *in vitro* (Youn et al., 2008). This compound can also inhibit *in vivo* and *in vitro* intracellular bacterial survival (specifically *A. actinomycetencomitans-LPS)* via autophagy activation, which was evidenced by the increase of autophagy markers (Beclin-1, ATG5, and LC3) (Chung et al., 2018).

Resveratrol is a type of natural phenol, particularly a stilbenoid, naturally produced by plants in response to stressors such as pathogens attack or ultraviolet radiation (Catalgol et al., 2012). Resveratrol can regulate autophagy-JNK signalling cascade in human dental pulp stem cells (DPSCs) model under TNFα-induced inflammation *in vitro* (Wang et al., 2018). When the focus is put on *in vivo* research there is no good evidence regarding its positive effects on human health. A recent randomized clinical trial (RCT) in which type 2 diabetic patients with PD were treated with scanning and root planning with or without a resveratrol supplementation evidenced the usefulness of resveratrol as an adjuvant therapy in this comorbidity (a significant improvement in pocket depth and insulin resistance, respectively) (Zare Javid et al., 2017).

Curcumin is a natural phenol belonging to the curcuminoids family. Curcumin is an unstable compound with limited bioavailability (Nelson et al., 2017). The ability to build rationale nanoparticles (NPs) has helped to explore curcumin-related pharmacokinetics in the management of several diseases (Beloqui et al., 2016). Curcumin can induce autophagy through inhibition of the Akt-mTOR pathway but also activating TFEB-lysosome pathway (Kocaadam and Sanlier, 2017). Curcumin can inhibit the bacterial growth by inhibiting A-type proton-pumping ATPase in the *P. gingivalis* membrane *in vitro* (Sekiya et al., 2018). Local administration of PLGA NPs loaded with curcumin has shown effectiveness in the treatment of experimental PD in mouse models (Zambrano et al., 2018). Muglikar et al. reported that a curcumin mouthrinse formulation was comparable to chlorhexidine as an adjunct to SRP in chronic PD affected patients in a clinical trial (Muglikar et al. 2013).

Melatonin is a hormone, mainly produced by the pineal gland. Nevertheless, naturally occurring melatonin can be found in several foods (Brzezinski, 1997). Melatonin acts on autophagy by the inhibition mTOR via AMPK activation, but it also can reverse H_2O_2-induced senescence via SIRT1-catalyzed deacetylation (Nopparat et al., 2017). Melatonin has been reported to have significant beneficial effects in animal models of PDs (Virto et al., 2018). Until today, two RCTs have ascertained the usefulness of

melatonin supplementation as a SRP adjuvant in PD (El-Sharkawy et al., 2018, Bazyar et al., 2018), and another RCT evaluated a melatonin-based topical formulation providing also positive results (Cutando et al., 2015).

3.4.2 Pharmaceutical drugs

Most described drugs that have potential as autophagy modulators for PDs present significant risk of unwanted effects and are not useful on the basis of a cost-benefit analysis (Preshaw, 2018). Bellow the most common pharmaceutical interferers with proven effectiveness on PDs are described. Chemical structures are shown in Fig. 3.

Rapamycin, also known as sirolimus, is a non-antibiotic macrolide that exhibits high immunosuppressive, and antiproliferative properties due to its ability to inhibit mTOR (Saxton and Sabatini, 2017). Back in 2009, Harrison et al. show that diet enriched with this compound prolongs lifespan of animals via caloric restriction and down-regulation of the insulin/insulin-like growth factor 1 (IGF-1) pathway (Harrison et al., 2009). This outcome is theoretically relevant to PD research, because aging increases disease susceptibility. Xia et al. demonstrated that sirolimus *in vitro* rejuvenates human gingival fibroblasts (HGFs) via ROS neutralization *in vitro* (Xia et al., 2017). An et al. showed that after three months of rapamycin treatment a reverse in age-associated alveolar bone loss could be reached in a mouse model (An et al., 2017).

Statins are HMG-CoA reductase inhibitors traditionally used as lipid-lowering drugs. They are highly used as primary and secondary coronary heart disease (CHD) prevention methods. Strong epidemiological reports have taken notice of the relationship between this outcome and PD, according to a systematic review performed by Humphrey this link pooled a relative risk estimation of 1.24 (95% confidence interval 1.01–1.51) (Humphrey et al., 2008). Statins act depleting cellular geranylgeranyl diphosphate, which ultimately produces AMPK activation and TOR inactivation (Araki and Motojima, 2008). Simvastatin both *in vivo* and *in vitro* provides a janus role in osteoblasts promoting autophagy and alleviating apoptosis (Lai et al., 2012). Local statin use as an adjunct to SRP has proven better results on PD managements that single SRP according to several trials (Bertl et al., 2017).

Vacuolar-type H^+-ATPases V-ATPases) are enzymes responsible for microenvironment acidification. Currently, a handful of V-ATPase inhibitors are known and represent potential therapeutic implications for several pathologies (Perez-Sayans et al., 2009). Acidic lysosomal microenvironment triggers autophagy. Nevertheless some V-ATPase inhibitors can use other mechanism to interfere on autophagy, specifically bafilomycin can knockdown autophagy via Ca-P60A/SERCA mediated autophagosome–lysosome fusion (Mauvezin et al., 2015). Bafilomycin A1 is a potent and selective inhibitor with well-known antibacterial, antifungal, antineoplastic, and immunosuppressive activities. In addition this drug has relevant a bone-related antiresorptive capacity (Gagliardi et al., 1999). A V-ATPases inhibitor has shown *in vitro* promising activities as PD treatment, specifically FR202126 (Niikura et al., 2005, Niikura, 2006). Back in 2008, our group discovered that intracellular pH regulation was mainly controlled by a single V-ATPase-related gene called ATP6V1C1 (Otero-Rey et al., 2008). Later, Li et al. by means of an *in vivo* model demonstrated that targeting this gene via adeno-associated virus-mediated Atp6v1c1 knockdown gene therapy prevented erosion and periodontal inflammation (Li et al., 2015).

Metformin is a biguanide clinically used in the treatment of non-insulin-dependent diabetes mellitus. Metformin exerts a biological function as AMPK activator and a secondary mTORC1 does not respond to the growth factor signals triggering gluconeogenesis inhibition via autophagy interference (Scheen et al., 2013). According to a recent systematic review of human trials better periodontal outcomes for SRP plus locally delivered metformin are obtained when compared to single SRP (Nicolini et al., 2018). In this line metformin is nowadays considered a geroprotector because it erects senescence barriers to inflammation.

3.5 Novel directions of autophagy research in PDs

Recently autophagy has been related to several more sophisticated issues in modern dentistry. In this sense, several authors are taking advantage of autophagic properties of several materials in the design of scaffolds for bone tissue regeneration (Li et al., 2016, Ozeki et al., 2017, Yin et al., 2018). Autophagy is necessary for the maintenance of cellular stemness and for osteogenic differentiation processes (Sbrana et al., 2016). Li et al. demonstrated that the inhibition of autophagy with 3-mathyladenine, bafilomycin A1, or NH4Cl before day 3 after placement of a fluorapatite-modified scaffold resulted

on a strong inhibition of osteogenesis and mineralization in a 3D cell culture (Li et al., 2016).

Several evidences linked cathepsins (Cts) to PD. Cathepsins are a family of proteases whose activity emerges at low lysosomal pH. The members of this family classically related to this family are CtsK, CtsC, and CtsS (Chen et al., 2016, Korkmaz et al., 2018, Memmert et al., 2017). *In vitro* research has proved that silencing CTsK by gene therapy silences periodontal inflammation (Chen et al., 2016). NADPH oxidase-derived superoxide mediates the activation of PNMs CtsG and neutrophil elastase *in vitro* (Reeves et al., 2002). Recently Bullon et al. in a landmark paper designed a recombinant CstC protein by means of baculovirus expression vector system (BEVS) coupled to an insect cell culture system. This protein was able to repair autophagic dysfunction in skin fibroblast isolated from Papillon-Lefèvre syndrome patients *in vitro* (Bullon et al., 2018). Papillon-Lefèvre syndrome is an extremely rare autosomal recessive genetic disorder characterised by a NET-driven severe destruction of periodontium (Van Dyke et al., 1984, Hahn et al., 2018).

A promising resource for further research is the modulation of autophagy at the level of saliva and salivary glands. Several autophagic dependent processes underlie salivary gland homeostasis and stress responses (Morgan-Bathke et al., 2015). Cut-edge research is working on the treatment of complex diseases with multiple target drugs. In this vein, the design of ideal molecules to fulfil the polypharmacologic needs of diseases such PDs are essential (Bastos et al., 2018). Antimicrobial salivary peptides (ASPs) play a significant role as immune systems protectors for PD-related disease drivers at the oral cavity and also serving in the discovery of new antimicrobial agents. Hypothetically, autophagy may be related to ASPs due to its capacity to enable cells to eliminate intracellular pathogens.

4. Conclusions

Autophagy underlies the biology of a significant portion of the current PD therapeutics. The understanding of the different subtypes of cell death and its interrelationships will mean a landmark in the discovery of novel PD-related HMTs and the optimization of the current ones, specifically the understanding of the balance between autophagy and apoptosis in periodontal tissues is a key issue. The present review highlights the

importance of nutrition in the periodontal health maintenance, due to the significance of several nutraceuticals in the autophagy pathway, as the right management of PD-related co-morbidities seems to provide better outcomes based on this relevant catabolic process. The potentiality of most autophagy interferers for PD has been examined only in preclinical trials, either in vitro or in vivo. Most of the current autophagy-based therapies rely on drugs far from being specific as they alter numerous cellular pathways other than autophagy and numerous pharmacokinetic challenges such as bioavailability, drug-drug interactions, and metabolic instability mean a significant shortcoming for clinical translation. The use of specific drugs in order to act on autophagy for PDs remains far-off.

Further basic research should explore this mechanism in order to reach clinical translation. Genetics should explore the possible presence of ATG-related polymorphism in the search of novel pharmacogenomics solutions. Transcriptomic studies may help to elucidate the ATG genes expression profiles and uncover novel transcriptional regulatory mechanisms of autophagy. Proteomic and metabolomic profiling can help to identify different environmental stresses and formulate ways to improve or modify cell fate on the basis of this type cell death. Future integrative multi-omics approaches will unravel the precise autophagy mechanism at a transcriptional, translational and post-translational level.

Figure legends:

Figure 1: The molecular machinery of autophagy. Adapted from Sbrana et al. 2016 and reproduced with permission from Springer Nature.

Autophagy initiation is regulated by the energy and nutrition central sensor, the mTOR kinase. Autophagy-Related 1 (ATG1)/ATG13 kinase complex directly works downstream target of rapamycin complex 1 (TORC1) pathway. mTORC1 regulates autophagy through serine/threonine-protein kinase (ULK1) protein phosphorylation. mTORC1 phosphorylation of ULK complex is regulated via autophagy/Beclin1 regulator 1 (AMBRA1). Protein kinase A (PKA) is a negative regulator of the ATG1/ATG13 kinase complex, while energy sensor snf1/AMP-activated protein kinase (AMPK) acts as a positive regulator.

Elongation refers to the amplification of the phagophore. In this process is involved the class III PI 3-kinase. Phagophore expansion is an Ubiquitin-like (UBL)-dependant process. In this vein, two UBL protein conjugation systems are needed in this process. The first ATG-related UBL conjugation system covalently binds to ATG12 and ATG5. Afterwards, this protein complex binds to ATG16L. The association of LC3-II to the nascent phagophore is orchestrated by the activity of the first UBL conjugation system. Once autophagosome formation is completed, soluble NSF attachment protein receptor (SNARE)-like proteins promote autophagosomes-lysosomes fusion. Finally, the contents of the autolysosome are degraded by various lysosomal enzymes at the lumen and released to the cytoplasm by permeases.

Figure 2: Chemical structures of natural products and/or derivatives able modulate autophagy in periodontal diseases (A) Maresin 1. (B) Genistein. (C) Resveratrol. (D) Curcumin. (E) Melatonin.

Figure 3: Chemical structures of pharmaceutical drugs derivatives able modulate autophagy in periodontal diseases (A) Rapamycin. (B) Statins [Simvastatin] (C) Bafilomycin A (D) Metformin.

TABLE 1: Reactive oxygen species (ROS) taken from Battino et al., 1999.

Reactive Oxygen Species			
Radicals		**Non radicals**	
Superoxide	O_2^-	Singlet oxygen	O_2
Hydroxyl	OH^-	Hidrogen peroxide	H_2O_2
Hydroperoxyl	HO_2	Hypochlorous acid	HOCl
Alkoxyl	RO^-	Hypobromous acid	HOBr
Aryloxyl	ArO^-	Ozone	O_3
Arylperoxyl	$ArOO^-$		
Peroxyl	ROO^-		
Acyloxyl	$RCOO^-$		
Acylperoxyl	$RCOOO^-$		

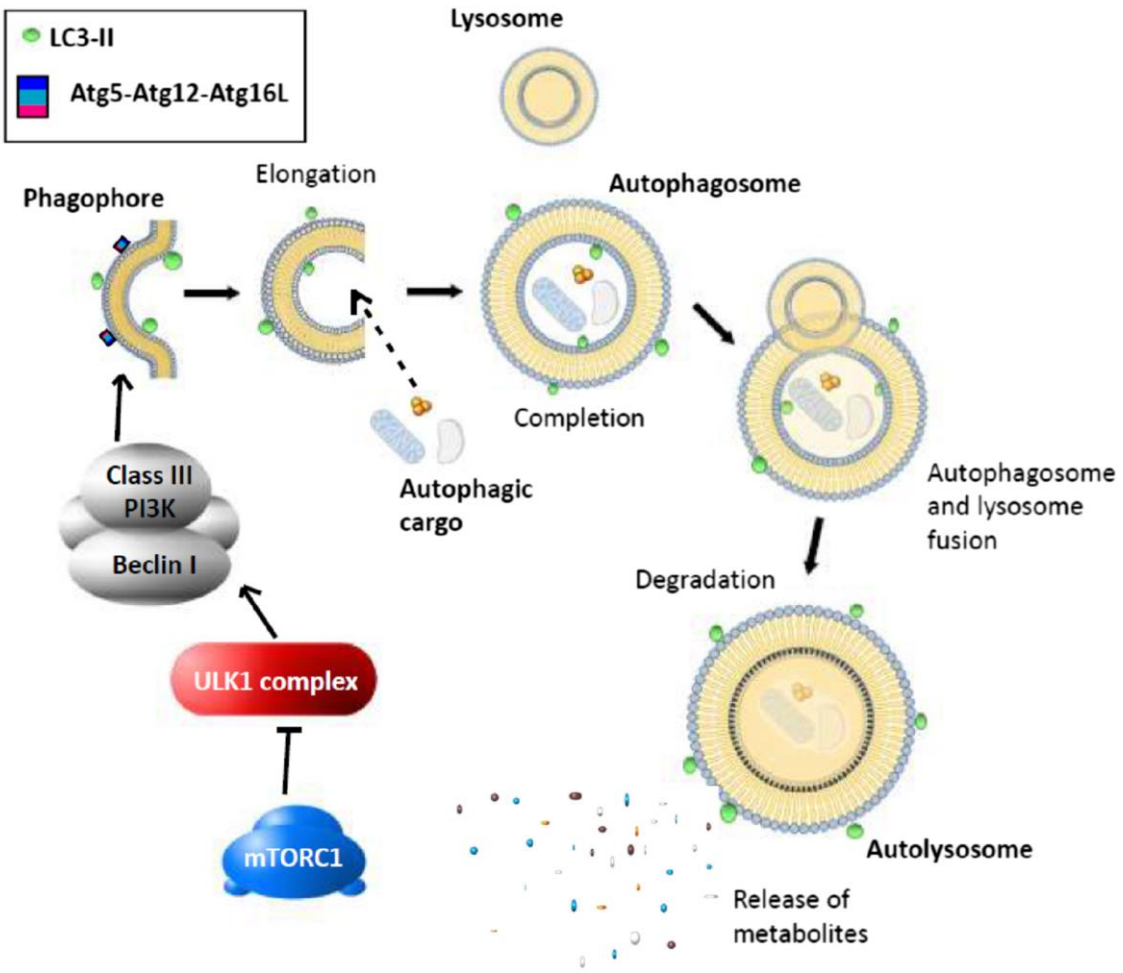
TABLE 2: Representative autophagy inducers and its interfering checkpoints. Original data was collected from Galluzzi et al. [41], and Giampieri et al. [5].

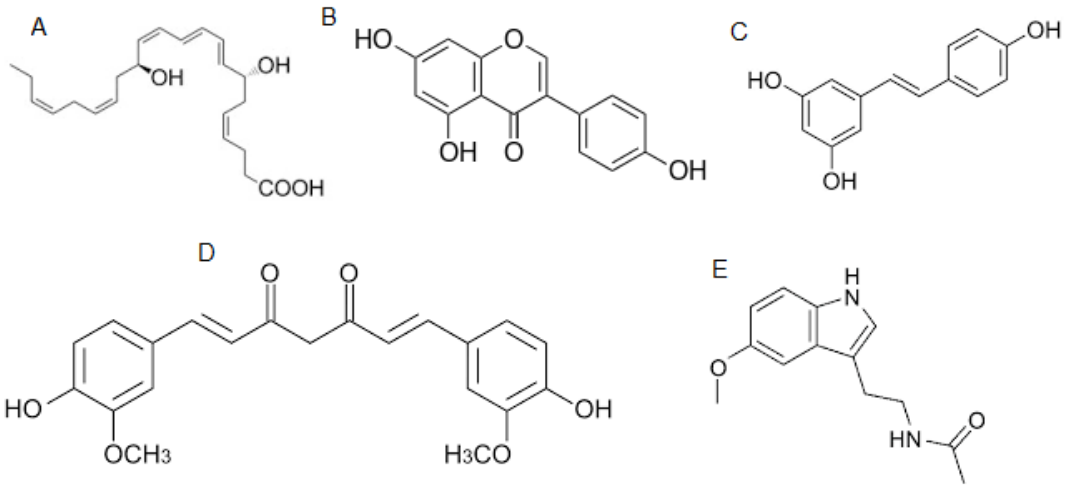
Autophagy inducers	**Mechanism of autophagy induction**
Drugs	
BH3 mimetics	Disrupt binding between beclin 1 and Bcl-2 family members
Clonidine	Lowers cAMP levels
Metformin	Upregulates AMPK, which phosphorylates ULK1 and beclin 1
Rapamycin (and rapalogs)	Inhibits mTORC1
Rilmenidine	Lowers cAMP levels
Sodium valproate	Lowers inositol and Ins(1,4,5)P3 levels
Verapamil	Inhibits L-type Ca2+ channel, lowering intracytosolic Ca2+
Tamoxifen	Increases sterol accumulation
Trifluoperazine	Unknown
Statins	Depletion of geranylgeranyl diphosphate activates AMPK
Tyrosine kinase inhibitors	Inhibit Akt-mTORsignaling and beclin 1 tyrosine phosphorylation, increase beclin 1/Parkin interaction
Bioactive compounds/ micronutrients	
Apigenin	Inhibits mTOR by AMPK activation
Berberine	Increases protein expression of LC3-II/LC3-I and Beclin-1
Caffeine	Inhibits mTORsignaling
Curcumin	Blocks the AKT-mTORsignaling pathway
Cinnamaldehyde	Upregulates AMPK, and downregulates AQP-1
Dioscin	Decreases phosphorylation of Akt and mTOR
Genistein	Inhibits Akt phosphorylation
Magnolol	Blocks the PI3K/PTEN/Akt pathway.
Omega-3 polyunsaturated fatty acids	Inhibits the Akt-mTORsignaling; disrupt beclin 1 and Bcl-2 binding

Resveratrol	Activates sirtuin 1
Selenium	Decreases Beclin-1 and LC3-II levels
Spermidine	Acetylase inhibitor

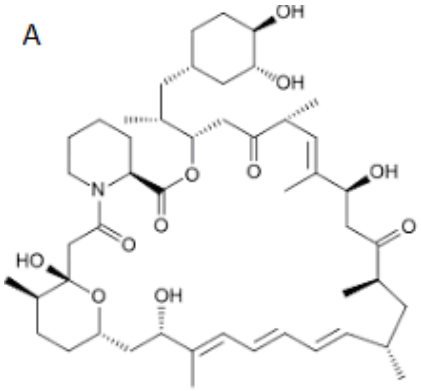
TABLE 3: Representative autophagy inhibitors and its interfering checkpoints. Original data was collected from Galluzzi et al. [41], and Giampieri et al. [5].

Autophagy inhibitors	**Mechanism of autophagy inhibition**
Drugs	
Hydroxychloroquine	Prevents lysosomal acidification
V-ATPase inhibitors	Knockdown of Atg5 and Beclin-1 expression
Vinblastine	Disrupts microtubules formation
Wortmannin (and derivatives)	Inhibits of PI-3 kinase
Others	
siRNAs	Multiple roles

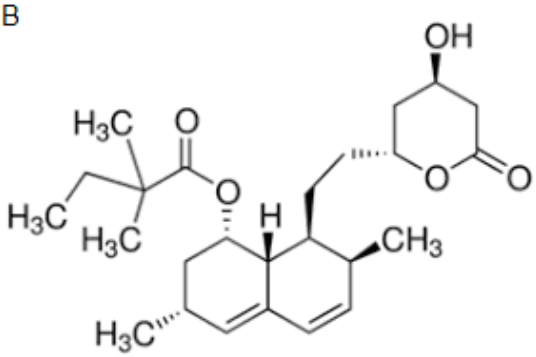

LC3-II
Atg5-Atg12-Atg16L
Lysosome
Phagophore
Elongation
Autophagosome
Completion
Autophagic cargo
Autophagosome and lysosome fusion
Degradation
Class III PI3K
Beclin I
ULK1 complex
mTORC1
Autolysosome
Release of metabolites


A
OH
OH
COOH
B
HO
O
OH
O
OH
C
OH
HO
OH
D
O
O
HO
OCH3
OH
H3CO
E
H
N
O
HN
O

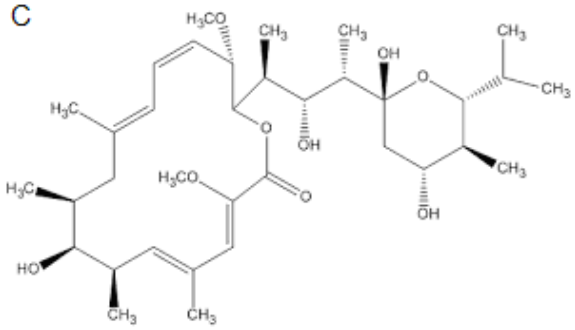
A



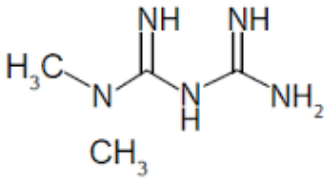
B



C



D



ACKNOWLEDGEMENTS

A.I. Lorenzo-Pouso is supported by a fellowship from the Health Research Institute of Santiago de Compostela (IDIS). Y. Leira holds a Senior Clinical Research Fellowship supported by the UCL Biomedical Research Centre (UK).

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Amano, A., Chen, C., Honma, K., Li, C., Settem, R. P., & Sharma A. (2014). Genetic characteristics and pathogenic mechanisms of periodontal pathogens. Advances in Dental Research, 26:15-22. https://doi.org/10.1177/0022034514526237.

An, J. Y., Quarles, E. K., Mekvanich, S., Kang A, Liu, A., Santos, D., Miller, R. A., Rabinovitch, P. S., Cox, T. C., & Kaeberlein, M. (2017). Rapamycin treatment attenuates age-associated periodontitis in mice. GeroScience. https://www.doi.org/10.1007/s11357-017-9994-6.

An, Y., Liu, W., Xue, P., Zhang, Y., Wang, Q., & Jin, Y. (2016). Increased autophagy is required to protect periodontal ligament stem cells from apoptosis in inflammatory microenvironment. Journal of clinical periodontology, 43:618-625. https://www.doi.org/10.1111/jcpe.12549.

Araki. M., & Motojima, K. (2008). Hydrophobic statins induce autophagy in cultured human rhabdomyosarcoma cells. Biochemical and biophysical research communications, 367:462-467. https://www.doi.org/10.1016/j.bbrc.2007.12.166.

Azzi, D. V., Viafara, J. A. S., Zangeronimo, M. G., Ribeiro Lima, R., Marques, L. S., & Pereira, L. J. (2018). n-3 Ingestion may modulate the severity of periodontal disease? Systematic review. Critical reviews in food science and nutrition, 58:1937-1942. https://www.doi.org/10.1080/10408398.2017.1278677.

Bastos, P., Trindade, F., da Costa, J., Ferreira, R., & Vitorino, R. (2018). Human Antimicrobial Peptides in Bodily Fluids: Current Knowledge and Therapeutic Perspectives in the Postantibiotic Era. Medicinal research reviews, 38:101-146. https://www.doi.org/10.1002/med.21435.

Battino, M., Bullon, P., Wilson, M., & Newman, H. (1999). Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. Critical reviews in oral biology and medicine, 10:458-476. https://doi.org/10.1177/10454411990100040301

Bazyar, H., Gholinezhad, H., Moradi, L., Salehi, P., Abadi, F., Ravanbakhsh, M., Zare & Javid, A. (2018). The effects of melatonin supplementation in adjunct with non-surgical periodontal therapy on periodontal status, serum melatonin and inflammatory markers in type 2 diabetes mellitus patients with chronic periodontitis: a double-blind, placebo-controlled trial. Inflammopharmacology. https://www.doi.org/10.1007/s10787-018-0539-0.

Beloqui, A., Memvanga, P. B., Coco, R., Reimondez-Troitino, S., Alhouayek, M., Muccioli, G. G., Alonso, M. J., Csaba, N., de la Fuente, M., & Preat, V. (2016). A comparative study of curcumin-loaded lipid-based nanocarriers in the treatment of inflammatory bowel disease. Colloids and surfaces B Biointerfaces, 143:327-335. https://www.doi.org/10.1016/j.colsurfb.2016.03.038.

Bertl, K., Parllaku, A., Pandis, N., Buhlin, K., Klinge, B., & Stavropoulos, A. (2017). The effect of local and systemic statin use as an adjunct to non-surgical and surgical periodontal therapy-A systematic review and meta-analysis. Journal of dentistry, 67:18-28. https://www.doi.org/ 10.1016/j.jdent.2017.08.011.

Bhattarai, G., Poudel, S. B., Kook, S. H., & Lee, J. C. (2017). Anti-inflammatory, anti-osteoclastic, and antioxidant activities of genistein protect against alveolar bone loss

and periodontal tissue degradation in a mouse model of periodontitis. Journal of biomedical materials research Part A, 105:2510-2521. https://www.doi.org/10.1002/jbm.a.36109.

Bostanci, N., & Belibasakis, G. N. (2012). Porphyromonas gingivalis: an invasive and evasive opportunistic oral pathogen. FEMS microbiology letters, 333:1-9. https://www.doi.org/10.1111/j.1574-6968.2012.02579.x.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., & Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. Science, 303:1532-1535. https://www.doi.org/ 10.1126/science.1092385.

Brzezinski, A. (1997). Melatonin in humans. The New England journal of medicine, 336:186-195. https://www.doi.org/10.1056/NEJM199701163360306.

Bullon, P., Castejon-Vega, B., Roman-Malo, L., Jimenez-Guerrero, M. P., Cotan, D., Forbes-Hernandez, T. Y., Varela-Lopez, A., Perez-Pulido, A. J., Giampieri, F., Quiles, J. L, Battino, M., Sanchez-Alcazar, J. A., & Cordero, M. D. (2018). Autophagic dysfunction in patients with Papillon-Lefevre syndrome is restored by recombinant cathepsin C treatment. The Journal of allergy and clinical immunology, 142:1131-1143.e7. https://www.doi.org/10.1016/j.jaci.2018.01.018.

Bullon, P., Cordero, M. D., Quiles, J. L., Ramirez-Tortosa, M. del C., Gonzalez-Alonso A., Alfonsi, S., Garcia-Marin, R., de Miguel, M., & Battino, M. (2012). Autophagy in periodontitis patients and gingival fibroblasts: unraveling the link between chronic diseases and inflammation. BMC medicine, 10:122-7015-10-122. https://www.doi.org/10.1186/1741-7015-10-122.

Bustos, V., Pulina, M. V., Kelahmetoglu, Y., Sinha, S. C., Gorelick, F. S., Flajolet, M., & Greengard, P. (2017). Bidirectional regulation of Abeta levels by Presenilin 1. Proceedings of the National Academy of Science of the United States of America, 114:7142-7147. https://www.doi.org/10.1073/pnas.1705235114.

Carlsson, G., Wahlin, Y.B., Johansson, A., Olsson, A., Eriksson, T., Claesson, R., Hänström, L., Henter, J.I. (2006) Periodontal disease in patients from the original Kostmann family with severe congenital neutropenia. Journal of Periodontology. 77:744-751. https://www.doi.org/10.1902/jop.2006.050191.

Catalgol, B., Batirel, S., Taga, Y., & Ozer, N. K. (2012). Resveratrol: French paradox revisited. Frontiers in pharmacology, 3, 141. https://www.doi.org/10.3389/fphar.2012.00141.

Chen, W., Gao, B, Hao, L., Zhu, G., Jules, J., MacDougall, M. J., Wang, J., Han, X., Zhou, X., & Li, Y. P. (2016). The silencing of cathepsin K used in gene therapy for periodontal disease reveals the role of cathepsin K in chronic infection and inflammation. Journal of periodontal research, 51:647-660. https://www.doi.org/10.1111/jre.12345.

Choi, E. Y., Bae, S. H., Ha, M. H., Choe, S. H., Hyeon, J. Y., Choi, J. I., Choi, I. S., & Kim, S. J. (2016). Genistein suppresses Prevotella intermedia lipopolysaccharide-induced inflammatory response in macrophages and attenuates alveolar bone loss in ligature-induced periodontitis. Archives of Oral Biology, 62:70-79. https://www.doi.org/10.1016/j.archoralbio.2015.11.019.

Chung, J., Kim, S., Lee, H.A., Park, M.H., Kim, S., Song, Y.R., Na, H.S. (2018). Trans-cinnamic aldehyde inhibits Aggregatibacter actinomycetemcomitans-induced inflammation in THP-1-derived macrophages via autophagy activation. Journal of Periodontology, 89:1262-1271. https://www.doi.org/10.1002/JPER.17-0727.

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. https://www.doi.org/10.1111/j.1600-0765.2006.00938.x.

Colotta, F., Re, F., Polentarutti, N., Sozzani, S., & Mantovani, A. (1992). Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood, 80:2012-2020. http://www.bloodjournal.org/content/80/8/2012.

Couve, E., Osorio, R., & Schmachtenberg, O. (2013). The amazing odontoblast: activity, autophagy, and aging. Journal of dental research, 92:765-772. https://www.doi.org/10.1177/0022034513495874.

Cuervo, A.M., & Wong, E (2014). Chaperone-mediated autophagy: roles in disease and aging. Cell research, 24:92-104. https://www.doi.org/10.1038/cr.2013.153.

Cutando, A., Montero, J., Gomez-de Diego, R., Ferrera, M. J., & Lopez-Valverde, A. (2015). Effect of topical application of melatonin on serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in patients with type 1 or type 2 diabetes and periodontal disease. Journal of clinical and experimental dentistry, 7: e628-33. https://www.doi.org/10.4317/jced.52604.

D'Aiuto, F., Gable, D., Syed, Z., Allen, Y., Wanyonyi, K. L., White, S., & Gallagher, J. E. (2017). Evidence summary: The relationship between oral diseases and diabetes. British dental journal, 222:944-948. https://www.doi.org/10.1038/sj.bdj.2017.544.

D'Aiuto, F., Gkranias, N., Bhowruth, D., Khan, T., Orlandi, M., Suvan, J., Masi, S., Tsakos, G., Hurel, S., Hingorani, A. D., Donos, N., Deanfield, J. E., & TASTE Group. (2018). Systemic effects of periodontitis treatment in patients with type 2 diabetes: a 12 month, single-centre, investigator-masked, randomised trial. The lancet Diabetes & endocrinology, 6:954-965. https://www.doi.org/10.1016/S2213-8587(18)30038-X.

D'Aiuto, F., Nibali, L., Parkar, M., Patel, K., Suvan, J., & Donos, N. (2010). Oxidative stress, systemic inflammation, and severe periodontitis. Journal of dental research, 89:1241-1246. https://www.doi.org/10.1177/0022034510375830.

Das Evcimen, N., & King, G. L. (2007). The role of protein kinase C activation and the vascular complications of diabetes. Pharmacological research, 55:498-510. https://www.doi.org/10.1016/j.phrs.2007.04.016.

da Silva, J. C., Muniz, F. W. M. G., Oballe, H. J. R., Andrades, M., Rosing, C. K., & Cavagni, J. (2018). The effect of periodontal therapy on oxidative stress biomarkers: A systematic review. Journal of clinical periodontology, 45:1222-1237. https://www.doi.org/10.1111/jcpe.12993.

Dommisch, H., Kuzmanova, D., Jonsson, D., Grant, M., & Chapple, I. (2018). Effect of micronutrient malnutrition on periodontal disease and periodontal therapy. Periodontology 2000, 78:129-153. https://www.doi.org/10.1111/prd.12233.

Du, L., Li, Y., & Liu, W. (2018). Maresin 1 regulates autophagy and inflammation in human periodontal ligament cells through glycogen synthase kinase-3beta/beta-catenin pathway under inflammatory conditions. Archives of Oral Biology, 87:242-247. https://www.doi.org/10.1016/j.archoralbio.2017.12.023.

Ebe, N., Hara-Yokoyama, M., Iwasaki, K., Iseki, S., Okuhara, S., Podyma-Inoue, K. A., Terasawa, K., Watanabe, A., Akizuki, T., Watanabe, H., Yanagishita, M., & Izumi, Y. (2011). Pocket epithelium in the pathological setting for HMGB1 release. Journal of dental research, 90: 235-240. https://www.doi.org/10.1177/0022034510385688.

El-Sharkawy, H., Elmeadawy, S., Elshinnawi, U., & Anees, M. (2018). Is dietary melatonin supplementation a viable adjunctive therapy for chronic periodontitis? A randomized controlled clinical trial. Journal of periodontal research, https://www.doi.org/10.1111/jre.12619.

Feng, J., Chen, X., & Shen, J. (2017). Reactive nitrogen species as therapeutic targets for autophagy: implication for ischemic stroke. Expert opinion on therapeutic targets, 21:305-317. https://www.doi.org/10.1080/14728222.2017.1281250.

Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V., & Zychlinsky, A. (2007). Novel cell death program leads to neutrophil extracellular traps. The Journal of cell biology, 176:231-241. https://www.doi.org/10.1083/jcb.200606027.

Gagliardi, S., Rees, M., & Farina, C. (1999). Chemistry and structure activity relationships of bafilomycin A1, a potent and selective inhibitor of the vacuolar H+-ATPase. Current medicinal chemistry, 6:1197-1212.

Galluzzi. L., Baehrecke, E. H., Ballabio, A., Boya, P., Bravo-San Pedro, J. M., Cecconi, F., Choi, A. M., Chu, C. T., Codogno, P., Colombo, M. I., Cuervo, A. M., Debnath, J., Deretic, V., Dikic, I., Eskelinen, E. L., Fimia, G. M., Fulda, S., Gewirtz, D. A., Green, D. R., Hansen, M., Harper, J. W., Jaattela, M., Johansen, T., Juhasz, G., Kimmelman, A C., Kraft, C., Ktistakis, N. T., Kumar, S., Levine, B., Lopez-Otin, C., Madeo, F., Martens, S., Martinez, J., Melendez, A., Mizushima, N., Munz, C., Murphy, L. O., Penninger, J. M., Piacentini, M., Reggiori, F., Rubinsztein, D. C., Ryan, K. M., Santambrogio, L., Scorrano, L., Simon, A. K., Simon, H. U., Simonsen, A., Tavernarakis, N., Tooze, S. A., Yoshimori, T., Yuan, J., Yue, Z., Zhong, Q., & Kroemer, G. (2017). Molecular definitions of autophagy and related processes. The EMBO journal, 36: 1811-1836. https://www.doi.org/10.15252/embj.201796697.

Galluzzi, L., Bravo-San Pedro, J. M., Levine, B., Green, D. R., & Kroemer, G. (2017). Pharmacological modulation of autophagy: therapeutic potential and persisting obstacles. Nature reviews Drug discovery, 16:487-511. https://www.doi.org/10.1038/nrd.2017.22.

Galluzzi, L., Vitale, I., Aaronson, S. A., Abrams, J. M., Adam, D., Agostinis, P., Alnemri, E. S., Altucci, L., Amelio, I., Andrews, D. W., Annicchiarico-Petruzzelli, M., Antonov, A. V., Arama, E., Baehrecke, E. H., Barlev, N. A., Bazan, N. G., Bernassola, F., Bertrand, M. J. M., Bianchi, K., Blagosklonny, M. V., Blomgren, K., Borner, C., Boya, P., Brenner, C., Campanella, M., Candi, E., Carmona-Gutierrez, D., Cecconi, F., Chan, F. K., Chandel, N. S., Cheng, E. H., Chipuk, J. E., Cidlowski, J. A., Ciechanover, A., Cohen, G. M., Conrad, M., Cubillos-Ruiz, J. R., Czabotar, P. E., D'Angiolella, V., Dawson, T. M., Dawson, V. L., De Laurenzi, V., De Maria, R., Debatin, K. M., DeBerardinis, R. J., Deshmukh, M., Di Daniele, N., Di Virgilio, F., Dixit, V. M., Dixon,

S. J., Duckett, C. S., Dynlacht, B. D., El-Deiry, W. S., Elrod, J. W., Fimia, G. M., Fulda, S., Garcia-Saez, A. J., Garg, A. D., Garrido, C., Gavathiotis, E., Golstein, P., Gottlieb, E., Green, D. R., Greene, L. A., Gronemeyer, H., Gross, A., Hajnoczky, G., Hardwick, J. M., Harris, I. S., Hengartner, M. O., Hetz, C., Ichijo, H., Jaattela, M., Joseph, B., Jost, .P J., Juin, P. P., Kaiser, W. J., Karin, M., Kaufmann, T., Kepp, O., Kimchi, A., Kitsis, R. N., Klionsky, D. J., Knight, R. A., Kumar, S., Lee, S. W., Lemasters, J. J., Levine, B., Linkermann, A., Lipton, S. A., Lockshin, R. A., Lopez-Otin, C., Lowe, S. W., Luedde, T., Lugli, E., MacFarlane, M., Madeo, F., Malewicz, M., Malorni, W., Manic, G., Marine, J. C., Martin, S. J., Martinou, J. C., Medema, J. P., Mehlen, P., Meier, P., Melino, S., Miao, E. A., Molkentin, J. D., Moll, U. M., Munoz-Pinedo, C., Nagata, S., Nunez, G., Oberst, A., Oren, M., Overholtzer, M., Pagano, M., Panaretakis, T., Pasparakis, M., Penninger, J. M., Pereira, D. M., Pervaiz, S., Peter, M. E., Piacentini. M., Pinton, P., Prehn, J. H. M., Puthalakath, H., Rabinovich, G. A., Rehm, M., Rizzuto, R., Rodrigues, C. M. P., Rubinsztein, D. C., Rudel, T., Ryan, K. M., Sayan, E., Scorrano, L., Shao, F., Shi, Y., Silke, J., Simon, H. U., Sistigu, A., Stockwell, B. R., Strasser, A., Szabadkai, G., Tait, S. W, G,, Tang, D., Tavernarakis, N., Thorburn, A., Tsujimoto. Y., Turk, B., Vanden Berghe, T., Vandenabeele, P., Vander Heiden, M. G., Villunger, A., Virgin, H. W, Vousden, K. H., Vucic, D., Wagner, E. F., Walczak, H., Wallach, D., Wang, Y., Wells, J. A., Wood, W., Yuan. J., Zakeri, Z., Zhivotovsky, B., Zitvogel, L., Melino, G., & Kroemer, G. (2018). Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell death and differentiation, 25:486-541. https://www.doi.org/10.1038/s41418-017-0012-4.

Genco, R.J. (1992). Host Responses in Periodontal Diseases: Current Concepts. Journal of periodontology, 63(Suppl 4S):338-355. https://www.doi.org/10.1902/jop.1992.63.4s.338.

Giampieri, F., Afrin, S., Forbes-Hernandez, T. Y., Gasparrini, M., Cianciosi, D., Reboredo-Rodriguez, P., Varela-Lopez, A., Quiles, J. L., & Battino, M. (2018). Autophagy in Human Health and Disease: Novel Therapeutic Opportunities. Antioxidants & redox signaling. https://www.doi.org/10.1089/ars.2017.7234.

Gil-Montoya, J.A., Barrios, R., Santana, S., Sanchez-Lara, I., Pardo, C. C., Fornieles-Rubio, F., Montes, J., Ramirez, C., Gonzalez-Moles, M. A., & Burgos, J. S. (2017). Association Between Periodontitis and Amyloid Beta Peptide in Elderly People with and Without Cognitive Impairment. Journal of periodontology, 88:1051-1058. https://www.doi.org/10.1902/jop.2017.170071.

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. The Journal of pathology, 221:3-12. https://www.doi.org/10.1002/path.2697.

Gonzalez-Mariscal, L., Betanzos, A., Nava, P., & Jaramillo, B. E. (2003). Tight junction proteins. Progress in biophysics and molecular biology, 81:1-44. https://doi.org/10.1016/S0079-6107(02)00037-8.

Goulhen, F., Grenier, D., & Mayrand, D. (2003). Oral microbial heat-shock proteins and their potential contributions to infections. Critical reviews in oral biology and medicine 14:399-412. https://doi.org/10.1177/154411130301400603.

Gutierrez, M.G., Master, S. S., Singh, S. B., Taylor, G. A., Colombo, M. I., & Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium

tuberculosis survival in infected macrophages. Cell, 119:753-766. https://doi.org/10.1016/j.cell.2004.11.038.

Hahn, J., Schauer, C., Czegley, C., Kling, L., Petru, L., Schmid, B., Weidner, D., Reinwald, C., Biermann, M. H. C., Blunder, S., Ernst, J., Lesner, A., Bauerle, T., Palmisano, R., Christiansen, S., Herrmann, M., Bozec, A., Gruber, R., Schett, G., & Hoffmann, M. H. (2018). Aggregated neutrophil extracellular traps resolve inflammation by proteolysis of cytokines and chemokines and protection from antiproteases. FASEB journal,33, fj201800752R. https://doi.org/10.1096/fj.201800752R.

Hajishengallis, E., & Hajishengallis, G. (2014). Neutrophil homeostasis and periodontal health in children and adults. Journal of dental research, 93:231-237. https://doi.org/10.1177/0022034513507956.

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, & Mizushima N (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature, 44:885-889. https://doi.org/10.1038/nature04724.

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., Nadon, N. L., Wilkinson, J. E., Frenkel, K., Carter, C. S., Pahor, M., Javors, M. A., Fernandez, E., & Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature, 460:392-395. https://doi.org/10.1038/nature08221.

Hazeldine, J., Harris, P., Chapple, I. L., Grant, M., Greenwood, H., Livesey, A., Sapey, E., & Lord, J. M. (2014). Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals. Aging cell, 13:690-698. https://doi.org/10.1111/acel.12222.

Hirschfeld, J., Roberts, H.M., Chapple, I.L., Parčina, M., Jepsen, S., Johansson, A., Claesson, R. (2016). Effects of Aggregatibacter actinomycetemcomitans leukotoxin on neutrophil migration and extracellular trap formation. Journal of Oral Microbiology. 8:33070. https://doi.org/10.3402/jom.v8.33070.

Hirschfeld, J., White, P. C., Milward, M. R., Cooper, P. R., & Chapple, I. L. C. (2017). Modulation of Neutrophil Extracellular Trap and Reactive Oxygen Species Release by Periodontal Bacteria. Infection and immunity, 85:10. https://doi.org/10.1128/IAI.00297-17.

Humphrey, L.L., Fu, R., Buckley, D. I., Freeman, M., & Helfand, M. (2008). Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. Journal of general internal medicine, 23:2079-2086. https://doi.org/10.1007/s11606-008-0787-6.

Ikenaka, K., Kawai, K., Katsuno, M., Huang, Z., Jiang, Y. M., Iguchi, Y., Kobayashi, K., Kimata, T., Waza, M., Tanaka, F., Mori, I., Sobue, G. (2013). Dnc-1/dynactin 1 Knockdown Disrupts Transport of Autophagosomes and Induces Motor Neuron Degeneration. PloS one, 8, e54511. https://doi.org/10.1371/journal.pone.0054511.

Jiang, M., Li, Z., Zhu, G. (2019). The role of autophagy in the pathogenesis of periodontal disease. Oral Diseases. https://www.doi.org/10.1111/odi.13045.

Kamer, A.R., Dasanayake, A. P., Craig, R. G., Glodzik-Sobanska, L., Bry, M., & de Leon, M. J. (2008). Alzheimer's disease and peripheral infections: the possible contribution from periodontal infections, model and hypothesis. Journal of Alzheimer's disease, 13:437-449. https://doi.org/10.3233/JAD-2008-13408.

Kamer, A.R., Pirraglia, E., Tsui, W., Rusinek, H., Vallabhajosula, S., Mosconi, L., Yi, L., McHugh, P., Craig, R. G., Svetcov, S., Linker, R., Shi, C., Glodzik, L., Williams, S., Corby, P., Saxena, D., & de Leon, M. J. (2015). Periodontal disease associates with higher brain amyloid load in normal elderly. Neurobiology of aging, 36:627-633. https://doi.org/10.1016/j.neurobiolaging.2014.10.038.

Kassebaum, N. J., Bernabe, E., Dahiya, M., Bhandari, B., Murray, C. J., & Marcenes, W. (2014). Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. Journal of dental research, 93:1045-1053. https://doi.org/10.1016/10.1177/0022034514552491.

Klionsky, D. J., & Emr, S. D. (2000). Autophagy as a regulated pathway of cellular degradation. Science, 290:1717-1721. http://dx.doi.org/10.1126/science.290.5497.1717.

Kocaadam, B., & Sanlier, N. (2017). Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Critical reviews in food science and nutrition, 57:2889-2895. https://doi.org/10.1080/10408398.2015.1077195.

Koori, K., Maeda, H., Fujii, S., Tomokiyo, A., Kawachi, G., Hasegawa, D., Hamano, S., Sugii, H., Wada, & N., Akamine, A. (2014). The roles of calcium-sensing receptor and calcium channel in osteogenic differentiation of undifferentiated periodontal ligament cells. Cell and tissue research, 357:707-718. https://doi.org/10.1007/s00441-014-1918-5.

Korkmaz, B., Caughey, G. H., Chapple, I., Gauthier, F., Hirschfeld, J., Jenne, D. E., Kettritz, R., Lalmanach, G., Lamort, A. S., Lauritzen, C., Legowska, M., Lesner, A., Marchand-Adam, S., McKaig, S. J., Moss, C., Pedersen, J., Roberts, H., Schreiber, A., Seren, S., & Thakker, N. S. (2018). Therapeutic targeting of cathepsin C: from pathophysiology to treatment. Pharmacology & therapeutics, 190:202-236. https://doi.org/10.1016/j.pharmthera.2018.05.011.

Lai, E. H., Hong, C. Y., Kok, S. H., Hou, K. L., Chao, L. H., Lin, L. D., Chen, M. H., Wu, P. H., & Lin, S. K. (2012). Simvastatin alleviates the progression of periapical lesions by modulating autophagy and apoptosis in osteoblasts. Journal of endodontics, 38:757-763. https://doi.org/10.1016/j.joen.2012.02.023.

Leira, Y., Dominguez, C., Seoane, J., Seoane-Romero, J., Pias-Peleteiro, J. M., Takkouche, B., Blanco, J., & Aldrey, J. M. (2017a). Is Periodontal Disease Associated with Alzheimer's Disease? A Systematic Review with Meta-Analysis. Neuroepidemiology, 48:21-31. https://doi.org/10.1159/000458411.

Leira, Y., Rodríguez-Yáñez, M., Arias, S., López-Dequidt, I., Campos, F., Sobrino, T., D'Aiuto, F., Castillo, J., Blanco, J. (2018). Periodontitis is associated with systemic inflammation and vascular endothelial dysfunction in patients with lacunar infarct. Journal of periodontology. https://www.doi.org/10.1002/JPER.18-0560.

Leira. Y., Seoane, J., Blanco, M., Rodriguez-Yanez, M., Takkouche, B., Blanco, J., & Castillo, J. (2017b). Association between periodontitis and ischemic stroke: a systematic

review and meta-analysis. European journal of epidemiology 32:43-53. https://doi.org/10.1007/s10654-016-0170-6.

Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. Cell, 132:27-42. https://doi.org/10.1016/j.cell.2007.12.018.

Levine, B., Packer, M., & Codogno, P. (2015). Development of autophagy inducers in clinical medicine. The Journal of clinical investigation, 125:14-24. https://doi.org/10.1172/JCI73938.

Levy, O. (2004). Antimicrobial proteins and peptides: anti-infective molecules of mammalian leukocytes. Journal of leukocyte biology, 76:909-925. https://doi.org/10.1189/jlb.0604320.

Li, S., Hao, L., Wang, L., Lu, Y., Li, Q., Zhu, Z., Shao, J. Z., & Chen, W. (2015). Targeting Atp6v1c1 Prevents Inflammation and Bone Erosion Caused by Periodontitis and Reveals Its Critical Function in Osteoimmunology. PloS one, 10, e0134903. https://doi.org/10.1371/journal.pone.0134903.

Li, Y., Guo, T., Zhang, Z., Yao, Y., Chang, S., Nor, J. E., Clarkson, B. H., Ni, L., & Liu, J. (2016). Autophagy Modulates Cell Mineralization on Fluorapatite-Modified Scaffolds. Journal of dental research, 95:650. https://doi.org/10.1177/0022034516636852.

Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., & Levine, B. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature, 402:672-676. https://doi.org/10.1038/45257.

Liu, C., Mo, L., Niu, Y., Li, X., Zhou, X., Xu, X. (2017). The Role of Reactive Oxygen Species and Autophagy in Periodontitis and Their Potential Linkage. Frontiers in Physiology 8:439. https://www.doi.org/10.3389/fphys.2017.00439.

Liu, J., Wang, X., Zheng, M., & Luan, Q. (2018). Lipopolysaccharide from Porphyromonas gingivalis promotes autophagy of human gingival fibroblasts through the PI3K/Akt/mTOR signaling pathway. Life Sciences, 211:133-139. https://doi.org/10.1016/j.lfs.2018.09.023.

Lopes, J.P., Stylianou, M., Backman, E., Holmberg, S., Jass, J., Claesson, R., Urban, C.F. (2018). Evasion of Immune Surveillance in Low Oxygen Environments Enhances Candida albicans Virulence. MBio. 9:02120-18. https://www.doi.org/10.1128/mBio.02120-18.

Luo, L. J., Liu, F., Lin, Z. K., Xie, Y. F., Xu, J. L., Tong, Q. C., & Shu, R. (2012). Genistein regulates the IL-1 beta induced activation of MAPKs in human periodontal ligament cells through G protein-coupled receptor 30. Archives of Biochemistry and Biophysics, 522: 9-16. https://doi.org/10.1016/j.abb.2012.04.007.

Mauvezin, C., Nagy, P., Juhasz, G., & Neufeld, T. P. (2015). Autophagosome-lysosome fusion is independent of V-ATPase-mediated acidification. Nature communications, 6:7007. https://doi.org/10.1038/ncomms8007.

Memmert, S., Damanaki, A., Nogueira, A. V. B., Eick, S., Nokhbehsaim, M., Papadopoulou, A. K., Till, A., Rath, B., Jepsen, S., Gotz, W., Piperi, C., Basdra, E. K., Cirelli, J. A., Jager, A., & Deschner, J. (2017). Role of Cathepsin S in Periodontal

Inflammation and Infection. Mediators of inflammation, 2017:4786170. https://doi.org/10.1155/2017/4786170.

Mitsuhashi, M., Yamaguchi, M., Kojima, T., Nakajima, R., & Kasai, K. (2011). Effects of HSP70 on the compression force-induced TNF-alpha and RANKL expression in human periodontal ligament cells. Inflammation research, 60:187-194. https://doi.org/10.1007/s00011-010-0253-x.

Mizushima, N. (2007). Autophagy: process and function. Genes & development, 21:2861-2873. https://doi.org/10.1101/gad.1599207

Mizushima, N., Yoshimori, T., & Levine, B. (2010). Methods in mammalian autophagy research. Cell, 140:313-326. https://doi.org/10.1016/j.cell.2010.01.028.

Monsarrat, P., Blaizot, A., Kemoun, P., Ravaud, P., Nabet, C., Sixou, M., Vergnes, J. N. (2016). Clinical research activity in periodontal medicine: a systematic mapping of trial registers. Journal of clinical periodontology, 43:390-400. https://doi.org/10.1111/jcpe.12534.

Morgan-Bathke, M., Lin, H. H., Ann, D. K., & Limesand, K. H. (2015). The Role of Autophagy in Salivary Gland Homeostasis and Stress Responses. Journal of dental research, 94:1035-1040. https://doi.org/10.1177/0022034515590796.

Moro, M.G., Silveira Souto, M. L., Franco, G. C. N., Holzhausen, M., & Pannuti, C. M. (2018). Efficacy of local phytotherapy in the nonsurgical treatment of periodontal disease: A systematic review. Journal of periodontal research, 53:288-297. https://doi.org/10.1111/jre.12525.

Muglikar, S., Patil, K.C., Shivswami, S., Hegde, R. (2013) Efficacy of curcumin in the treatment of chronic gingivitis: a pilot study. Oral Health and Preventive Dentistry. 11:81-6. https://doi.org/10.3290/j.ohpd.a29379.

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., Hamada, S., & Yoshimori, T. (2004). Autophagy defends cells against invading group A Streptococcus. Science, 306:1037-1040. https://doi.org/10.1126/science.1103966

Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The Essential Medicinal Chemistry of Curcumin. Journal of medicinal chemistry, 60:1620-1637. https://doi.org/10.1021/acs.jmedchem.6b00975.

Nicolini, A.C., Grisa, T.A., Muniz, F.W.M.G., Rösing, C.K., Cavagni, J. (2018) Effect of adjuvant use of metformin on periodontal treatment: a systematic review and meta-analysis. https://doi.org/10.1007/s00784-018-2666-9.

Niikura, K. (2006). Comparative analysis of the effects of a novel vacuolar adenosine 5'-triphosphatase inhibitor, FR202126, and doxycycline on bone loss caused by experimental periodontitis in rats. Journal of periodontology, 77:1211-1216. https://doi.org/10.1902/jop.2006.050344.

Niikura, K., Takeshita, N., & Chida, N. (2005). A novel inhibitor of vacuolar ATPase, FR202126, prevents alveolar bone destruction in experimental periodontitis in rats. The Journal of toxicological sciences, 30:297-304. https://doi.org/10.2131/jts.30.297.

Nixon, R.A., Wegiel, J., Kumar, A., Yu, W. H., Peterhoff, C., Cataldo, A., & Cuervo, A. M. (2005). Extensive involvement of autophagy in Alzheimer disease: an immuno-

electron microscopy study. Journal of neuropathology and experimental neurology, 64:113-122. https://doi.org/10.1093/jnen/64.2.113.

Nopparat, C., Sinjanakhom, P., & Govitrapong, P. (2017). Melatonin reverses H2 O2 - induced senescence in SH-SY5Y cells by enhancing autophagy via sirtuin 1 deacetylation of the RelA/p65 subunit of NF-kappaB. Journal of pineal research, 63. https://doi.org/10.1111/jpi.12407.

Nussbaum, G., Shapira, L. (2011). How has neutrophil research improved our understanding of periodontal pathogenesis? Journal of clinical periodontology, 38(Suppl11):49-59. https://doi.org/10.1111/j.1600-051X.2010.01678.x.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., & Sasakawa, C. (2005). Escape of intracellular Shigella from autophagy. Science, 307:727-731. https://doi.org/10.1126/science.1106036.

Otero-Rey, E. M., Somoza-Martin, M., Barros-Angueira, F., & Garcia-Garcia, A. (2008). Intracellular pH regulation in oral squamous cell carcinoma is mediated by increased V-ATPase activity via over-expression of the ATP6V1C1 gene. Oral oncology, 44:193-199. https://doi.org/10.1016/j.oraloncology.2007.02.011.

Ozeki, N., Hase, N., Higuchi, N., Hiyama, T., Yamaguchi, H., Kawai, R., Matsumoto, T., Nakata, K., & Mogi, M. (2017). Gelatin scaffold combined with bone morphogenetic protein-4 induces odontoblast-like cell differentiation involving integrin profile changes, autophagy-related gene 10, and Wnt5 sequentially in human induced pluripotent stem cells. Differentiation, 93:1-14. https://doi.org/10.1016/j.diff.2016.09.002.

Page, R.C., & Kornman, K. S. (1997). The pathogenesis of human periodontitis: an introduction. Periodontology 2000, 14:9-11. https://doi.org/10.1111/j.1600-0757.1997.tb00189.x.

Papayannopoulos, V. (2014). Infection: microbial nucleases turn immune cells against each other. Current biology, 123-5. https://doi.org/10.1016/j.cub.2013.12.027.

Perez-Gregorio, R., & Simal-Gandara, J. (2017). A Critical Review of Bioactive Food Components, and of their Functional Mechanisms, Biological Effects and Health Outcomes. Current pharmaceutical design, 23:2731-2741. https://doi.org/10.2174/1381612823666170317122913.

Perez-Sayans, M., Somoza-Martin, J. M., Barros-Angueira, F., Rey, J. M., & Garcia-Garcia, A. (2009). V-ATPase inhibitors and implication in cancer treatment. Cancer treatment reviews, 35:707-713. https://doi.org/10.1016/j.ctrv.2009.08.003.

Preshaw, P. M. (2018). Host modulation therapy with anti-inflammatory agents. Periodontology 2000, 76:131-149. https://doi.org/10.1111/prd.12148.

Pritchard, A. B., Crean, S., Olsen, I., & Singhrao, S. K. (2017). Periodontitis, Microbiomes and their Role in Alzheimer's Disease. Frontiers in aging neuroscience, 9:336. https://doi.org/10.3389/fnagi.2017.00336.

Pussinen, P.J., Tuomisto, K., Jousilahti, P., Havulinna, A.S., Sundvall, J., Salomaa, V. (2007). Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. Arteriosclerosis,

thrombosis, and vascular biology, 24:1433-1439. https://www.doi.org/10.1161/ATVBAHA.106.138743.

Pussinen, P.J., Vilkuna-Rautiainen, T., Alfthan, G., Palosuo, T., Jauhiainen, M., Sundvall, J., Vesanen, M., Mattila, K., Asikainen, S. (2004). Severe periodontitis enhances macrophage activation via increased serum lipopolysaccharide. Arteriosclerosis, thrombosis, and vascular biology, 24:2174-2180. https://www.doi.org/10.1161/01.ATV.0000145979.82184.9f.

Reaven, M. G. (1988). Banting lecture 1988. Role of insulin resistance in human disease. Diabetes, 37:1595-1607. https://doi.org/10.2337/diab.37.12.1595.

Reeves, E.P., Lu, H., Jacobs, H. L., Messina, C. G., Bolsover, S., Gabella, G., Potma, E. O., Warley, A., Roes, J., & Segal, A. W. (2002). Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature, 416:291-297. https://doi.org/10.1038/416291a.

Reitz, C., Brayne, C., & Mayeux, R. (2011). Epidemiology of Alzheimer disease. Nature reviews Neurology 7:137-152. https://doi.org/10.1038/nrneurol.2011.2.

Ritchie, C. S., Joshipura, K., Hung, H. C., & Douglass, C. W. (2002). Nutrition as a mediator in the relation between oral and systemic disease: associations between specific measures of adult oral health and nutrition outcomes. Critical reviews in oral biology and medicine 13:291-300. https://doi.org/10.1177/154411130201300306.

Rubinsztein, D. C., Codogno, P., & Levine, B. (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. Nature reviews Drug discovery, 11:709-730. https://doi.org/10.1038/nrd3802.

Sachdev, U., & Lotze, M. T. (2017). Perpetual change: autophagy, the endothelium, and response to vascular injury. Journal of leukocyte biology, 102:221-235. https://doi.org/10.1189/jlb.3RU1116-484RR.

Sahu, R., Kaushik, S., Clement, C. C., Cannizzo, E. S., Scharf, B., Follenzi, A., Potolicchio, I., Nieves, E., Cuervo, A. M., & Santambrogio, L. (2011). Microautophagy of cytosolic proteins by late endosomes. Developmental cell, 20:131-139. https://doi.org/10.1189/10.1016/j.devcel.2010.12.003.

Sbrana, F.V., Cortini, M. Avnet, S., Perut, F., Columbaro, M., De Milito, A., & Baldini, N. (2016). The Role of Autophagy in the Maintenance of Stemness and Differentiation of Mesenchymal Stem Cells. Stem cells reviews and reports, 12: 621-633. https://doi.org/10.1007/s12015-016-9690-4.

Scheen, A.J., Paquot, N. (2013) Metformin revisited: A critical review of the benefit-risk balance in at-risk patients with type 2 diabetes. Diabetes Metabolism. 39:179–90. https://doi.org/10.1016/j.diabet.2013.02.006.

Sekiya, M., Shimoyama, Y., Ishikawa, T., Sasaki, M., Futai, M., & Nakanishi-Matsui, M. (2018). Porphyromonas gingivalis is highly sensitive to inhibitors of a proton-pumping ATPase. Biochemical and biophysical research communications, 498:837-841. https://doi.org/10.1016/j.bbrc.2018.03.066.

Shi, W., Wei, X., Wang, Z., Han, H., Fu, Y., Liu, J., Zhang, Y., Guo, J., Dong, C., Zhou, D., Zhou, Q., Chen, Y., & Yi, F. (2016). HDAC9 exacerbates endothelial injury

in cerebral ischaemia/reperfusion injury. Journal of Cellular and Molecular Medicine, 20:1139-1149. https://doi.org/10.1111/jcmm.12803.

Socransky, S.S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L., Jr. (1998). Microbial complexes in subgingival plaque. Journal of clinical periodontology, 25(2): 134-144. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x.

Stefano, G. B., Challenger, S., & Kream, R. M. (2016). Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic disorders. European journal of nutrition, 55:2339-2345. https://doi.org/10.1007/s00394-016-1212-2

Takeshige, K., Baba, M., Tsuboi, S., Noda, T., & Ohsumi, Y. (1992). Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. The Journal of cell biology, 119:301-311. https://doi.org/10.1083/jcb.119.2.301

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. https://doi.org/10.1111/j.1462-5822.2010.01564.x.

Tan, Y. Q., Zhang, J., & Zhou, G. (2017). Autophagy and its implication in human oral diseases. Autophagy, 13:225-236. https://doi.org/10.1080/15548627.2016.1234563.

Tsuda, H., Ning, Z., Yamaguchi, Y., & Suzuki, N. (2012). Programmed cell death and its possible relationship with periodontal disease. Journal of oral science, 54:137-149. https://doi.org/10.2334/josnusd.54.137.

Tsuda, H., Ochiai, K., Suzuki, N., & Otsuka, K. (2010). Butyrate, a bacterial metabolite, induces apoptosis and autophagic cell death in gingival epithelial cells. Journal of periodontal research, 45:626-634. https://doi.org/10.1111/j.1600-0765.2010.01277.x.

Van Dyke, T. E., Taubman, M. A., Ebersole, J. L., Haffajee, A. D., Socransky, S. S., Smith, D. J., & Genco, R. J. (1984). The Papillon-Lefevre syndrome: neutrophil dysfunction with severe periodontal disease. Clinical immunology and immunopathology 31:419-429. https://doi.org/10.1016/0090-1229(84)90094-1

Van Dyke, T. E., & van Winkelhoff, A. J. (2013). Infection and inflammatory mechanisms. Journal of clinical periodontology 40 (Suppl14): S1-7. https://doi.org/10.1111/jcpe.12088.

Varela-Lopez, A., Bullon, P., Giampieri, F., & Quiles, J. L. (2015). Non-Nutrient, Naturally Occurring Phenolic Compounds with Antioxidant Activity for the Prevention and Treatment of Periodontal Diseases. Antioxidants, 4:447-481. https://doi.org/10.3390/antiox4030447.

Varela-Lopez, A., Navarro-Hortal, M. D., Giampieri, F., Bullon, P., Battino, M., & Quiles, J. L. (2018). Nutraceuticals in Periodontal Health: A Systematic Review on the Role of Vitamins in Periodontal Health Maintenance. Molecules, 23. https://doi.org/10.3390/molecules23051226.

Virto, L., Haugen, H. J., Fernandez-Mateos, P., Cano, P., Gonzalez, J., Jimenez-Ortega, V., Esquifino, A. I., & Sanz, M. (2018). Melatonin expression in periodontitis and obesity: An experimental in-vivo investigation. Journal of periodontal research 53:825-831. https://doi.org/10.1111/jre.12571.

Waddington, R.J., Moseley, R., & Embery, G. (2000). Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral diseases, 6:138-151.

Wang, C.W., Colas, R. A., Dalli, J., Arnardottir, H. H., Nguyen, D., Hasturk, H., Chiang, N., Van Dyke, T. E., & Serhan, C. N. (2015). Maresin 1 Biosynthesis and Proresolving Anti-infective Functions with Human-Localized Aggressive Periodontitis Leukocytes. Infection and immunity, 84:658-665. https://doi.org/10.1128/IAI.01131-15.

Wang, F. M., Hu, Z., Liu, X., Feng, J. Q., Augsburger, R. A., Gutmann, J. L., & Glickman, G. N. (2018). Resveratrol represses tumor necrosis factor alpha/c-Jun N-terminal kinase signaling via autophagy in human dental pulp stem cells. Archives of Oral Biology, 97:116-121. https://doi.org/10.1016/j.archoralbio.2018.10.020.

Warlow, C.P. (1998). Epidemiology of stroke. Lancet, 352(Suppl 3), SIII1-4. https://doi.org/10.1016/10.1016/S0140-6736(98)90086-1.

Wei, W., An, Y., An, Y., Fei, D., & Wang, Q. (2018). Activation of autophagy in periodontal ligament mesenchymal stem cells promotes angiogenesis in periodontitis. Journal of periodontology, 89:718-727. https://doi.org/10.1002/JPER.17-0341.

White, P. C. Chicca, I. J., Cooper, P. R., Milward, M. R., & Chapple, I. L. (2016). Neutrophil Extracellular Traps in Periodontitis: A Web of Intrigue. Journal of dental research, 95:26-34. https://doi.org/10.1177/0022034515609097.

Wolf, M., Marciniak, J., Lossdorfer, S., Kirschneck, C., Brauner, I., Gotz, W., & Jager, A. (2019). Role of HSP70 protein in human periodontal ligament cell function and physiology. Annals of Anatomy, 221:76-83. https://doi.org/10.1016/j.aanat.2018.09.006.

Xia, Y., Sun., M, Xie, Y., & Shu, R. (2017). mTOR Inhibition Rejuvenates the Aging Gingival Fibroblasts through Alleviating Oxidative Stress. Oxidative medicine and cellular longevity, 2017:6292630. https://doi.org/10.1155/2017/6292630.

Xu, F., Zhang, C., Zou, Z., Fan, E. K. Y., Chen, L., Li, Y., Billiar, T. R. Wilson, M. A., Shi, X., & Fan, J. (2017). Aging-related Atg5 defect impairs neutrophil extracellular traps formation. Immunology, 151:417-432. https://doi.org/10.1111/imm.12740.

Yin, J., Gong, G., Sun, C., Yin, Z., Zhu, C., Wang, B., Hu, Q., Zhu, Y., & Liu, X. (2018). Angiopoietin 2 promotes angiogenesis in tissue-engineered bone and improves repair of bone defects by inducing autophagy. Biomedicine & pharmacotherapy, 105:932-939. https://doi.org/10.1016/j.biopha.2018.06.078.

Yoon, Y., Kim, T. J., Lee, J. M., & Kim, D. Y. (2018). SOD2 is upregulated in periodontitis to reduce further inflammation progression. Oral diseases, 24:1572-1580. https://doi.org/10.1016/10.1111/odi.12933.

Youn, H.S., Lee, J.K., Choi, Y.J., Saitoh, S.I., Miyake, K., Hwang, D.H., Lee, J.Y. (2008). Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization. Biochemistry Pharmacology. 75:494-502. https://doi.org/10.1016/j.bcp.2007.08.033.

Zambrano, L. M. G., Brandao, D. A., Rocha, F. R. G., Marsiglio, R. P., Longo, I. B., Primo, F. L., Tedesco, A. C., Guimaraes-Stabili, M. R., & Rossa Junior, C. (2018). Local administration of curcumin-loaded nanoparticles effectively inhibits inflammation

and bone resorption associated with experimental periodontal disease. Scientific reports, 8:6652-018-24866-2. https://doi.org/10.1038/s41598-018-24866-2.

Zare Javid, A., Hormoznejad, R., Yousefimanesh, H. A., Zakerkish, M., Haghighi-Zadeh, M. H., Dehghan, P., & Ravanbakhsh, M. (2017). The Impact of Resveratrol Supplementation on Blood Glucose, Insulin, Insulin Resistance, Triglyceride, and Periodontal Markers in Type 2 Diabetic Patients with Chronic Periodontitis. Phytotherapy Research, 31:108-114. https://doi.org/10.1002/ptr.5737.

Zhang, B., Hou, R., Zou, Z., Luo, T., Zhang, Y., Wang, L., & Wang, B. (2018). Mechanically induced autophagy is associated with ATP metabolism and cellular viability in osteocytes in vitro. Redox biology, 14:492-498. https://doi.org/10.1016/j.redox.2017.10.021.

Zhang, Y., Du, Y., Le, W., Wang, K., Kieffer, N., & Zhang, J. (2011). Redox control of the survival of healthy and diseased cells. Antioxidants & redox signaling, 15:2867-2908. https://doi.org/10.1089/ars.2010.3685.

Zheng, L., Terman, A., Hallbeck, M., Dehvari, N., Cowburn, R. F., Benedikz, E., Kagedal, K., Cedazo-Minguez, A., & Marcusson, J. (2011). Macroautophagy-generated increase of lysosomal amyloid beta-protein mediates oxidant-induced apoptosis of cultured neuroblastoma cells. Autophagy, 7:1528-1545. https://doi.org/10.4161/auto.7.12.18051.

van Beek, N., Klionsky, D. J., & Reggiori, F. (2018). Genetic aberrations in macroautophagy genes leading to diseases. Biochimica et biophysica acta, 1865:803-816. https://doi.org/10.1016/j.bbamcr.2018.03.002.