Endoplasmic Reticulum and Its Significance in Periodontal Disease

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The endoplasmic reticulum has emerged as a modulator that is essential for cellular homeostasis and human health. It is an extensive membranous organelle that acts as a hub for the physiological and pathological processes. In recent years, it has become a topic of interest in studies on the relationship between endoplasmic reticulum homeostasis and system diseases. Periodontal disease is a prevalent chronic disease that affects tooth-supporting tissues, initiated by the interaction between pathogenic bacterial infection and immune defence and resulting in tooth loss. The endoplasmic reticulum participates in the responses to the fluctuating microenvironments in periodontal pathogenesis and regulates periodontal homeostasis. In this review, we present an overview of the significance of endoplasmic reticulum regulation as a multidimensional mediator in periodontal disease and highlight the potential strategies for periodontal regeneration.

Key words: endoplasmic reticulum, periodontal disease, periodontal regeneration

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The periodontium is a highly organised group of tissues that support the teeth and consists of gingival tissue, the cementum, the periodontal ligament (PDL) and alveolar bone¹. As an important complex structure that is essential for maintaining tooth stability, the periodontium undergoes constant remodelling throughout life in various microenvironments². Periodontal diseases are highly prevalent worldwide and comprise a wide variety of inflammatory conditions of the periodontium³. This kind of disease begins with localised inflammation of the gingiva, known as gingivitis. If the inflammation persists, untreated gingivitis may progress to periodontitis, characterised by destruction of the PDL and alveolar bone resorption, and eventually lead to tooth loss. Interestingly, periodontitis correlates with several system disorders, such as diabetes and hypertension⁴,⁵.

In recent years, regulation of the endoplasmic reticulum (ER) has attracted remarkable attention in relation to many diseases, such as diabetes and neurodegenerative and inflammatory diseases⁶-⁸. Meanwhile, accumulating evidence has demonstrated that ER is a multidimensional modulator in the pathogenesis of periodontal disease. Currently identified as a sophisticated system that mediates an array of biosynthetic signalling processes and organelle dynamics⁹-¹¹, the critical function of ER in determining periodontal modelling continues to be revealed. ER compromise has further demonstrated impacts on the proliferation, differentiation and apoptosis of stem cells¹². Accordingly, medication targeting the ER may support dental tissue regeneration,
aiming to further elucidate the contribution of the ER to periodontal homeostasis, disorders and regeneration.

The ER: A hub of physiological and pathological processes

The ER is a membranous organelle, a large hub that extends from the nuclear membrane to the plasma membrane, establishing a certain connection with other organelles such as mitochondria, the Golgi apparatus and lysosomes. It is divided into the smooth ER and rough ER, which are distinguished according to whether there are (granular) ribosomes on the envelope.

The ER is involved in calcium storage responsible for intracellular calcium homeostasis and secreted proteins are synthesised and folded there. ER homeostasis can be disrupted by diverse environmental stresses including hypoxia, oxidative stress, malnutrition and intracellular pathogens, under which circumstances the cells carry out a series of adaptive and protective reactions to overcome the ER stress, called unfolded protein response (UPR). UPR is a complex and coordinated cellular response. It is regulated by three transmembrane receptors in the endoplasmic reticulum: protein kinase receptor-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1), also known as ERN1. It is essential for alleviating cellular stress, which accurately responds to reduce the accumulation of unfolded proteins and restore the normal function of the ER. Beyond ER proteostasis, it also participates in various biological processes such as autophagy, cytoskeleton dynamics and reactive oxygen species (ROS) production. Additionally, ER communicates with other organelles, in which it mediates the regulation of mitochondria dynamics, cellular calcium oscillations and autophagosome formation.

Association of ER with periodontal disease

Previous genome-wide associated studies have found that the ER membrane as the top gene set is correlated with severe periodontitis. Levels of UPR-related gene expression are higher in periodontitis lesions than in gingivitis lesions. Additionally, a study found the percentage of cells expressing heat shock protein 60 (HSP60), chaperones of intracellular proteins during protein folding, was greater in the periodontitis group than the gingivitis group. Russell bodies (RBs) are indigested proteins accumulated in dilated ER, which correlates with ER occurrence in periodontal inflammation. Furthermore, dilated ER and upregulated UPR genes have been found in PDL stem cells (PDLSCs) isolated from periodontitis patients.

The ER is notably related to risk factors for periodontal disease. Smoking is considered one of the major risk factors for periodontitis. Nicotine-induced cytotoxicity and extracellular matrix (ECM) degradation by matrix metalloproteinases (MMPs) in human PDL cells (hPDLs) have been proven to be mediated by the ER stress pathway. Diabetes is the most studied systemic disease that predisposes periodontitis. ER stress could be induced by advanced glycation end products (AGEs) in PDLSCs. In high glucose concentrations, proteomic analysis of PDL fibroblasts (PDLFs) revealed an increased abundance of chaperonins and E3 ubiquitin ligases involved in misfolded protein degradation. Force-induced cell death is also an important mediator of periodontal connective tissue destruction. PERK has been proven to mediate force-induced apoptosis in mouse embryonic fibroblasts. Mechanical strain could downregulate cell viability and increase the level of expression of UPR-related genes. Ageing is also a trigger of alveolar bone resorption. The expression of several ER stress markers is higher in osteocytes from bones of old compared to adult mice.

ER regulation in the pathogenesis of periodontal disease

As examples of local inflammation, chronic gingivitis and periodontitis are triggered and sustained by periodontopathic bacteria. Porphyromonas gingivalis may be the indicative species for progressive periodontitis, while Aggregatibacter actinomycetemcomitans are associated with aggressive forms of periodontal disease. Meanwhile, it is widely recognised that the interaction between subgingival dental plaque and the host immune response contributes to the occurrence and development of periodontitis. In the following section, we describe how periodontal pathogens interact with the ER, and how the latter contributes to immune responses against invading pathogens. In such situations, the ER plays a significant role in local inflammation and indeed homeostasis in the entire organism.

ER responses to periodontal pathogen invasion

Human primary gingival epithelial cells (GECs) are the first line of oral mucosa defence, which is vital for the success of pathogen invasion. Porphyromonas gingivalis (P. gingivalis) is closely associated with ER structures and exploits ER networks as an excellent subcellular niche for intracellular location in human GECs. The following investigation studied the intracellular trafficking of
P. gingivalis in GECs and revealed that a large quantity of P. gingivalis localise to perinuclear ER regions and are encapsulated in ER-rich/LC3-positive double membrane vacuoles. As a result, P. gingivalis might utilise ER-rich autophagosomes to escape from antimicrobial mechanisms. ER-associated degradation (ERAD) is a physiological process that transfers misfolded proteins from the ER lumen and membrane to the cytoplasm for further degradation by the proteasome. The components of the ERAD pathway are implicated in host cell entry and the toxicity of A. actinomycetemcomitans cytotoxic distending toxin (AaCDT). Consistently, several ER-associated genes were found to be required for AaCDT toxicity in genome-wide screening in a yeast model.

Bacterial toxins can also induce UPR. Lipopolysaccharide (LPS), a component of gram-negative bacteria, has been applied widely to simulate the process of periodontal pathogen stimulation in vitro. The ER stress signalling pathway was activated by P. gingivalis LPS stimuli in hPDLCs. Beyond the local invasion, oral administration of P. gingivalis significantly increased expression of glucose-regulated protein 78 (GRP78) and IRE1α/X-box binding protein-1 (XBP1) participating in UPR pathways was detected in the gingival tissue.

**ER and periodontal immune response**

The invasion of pathogens elicits immune responses, dedicated to killing bacteria with immune cell infiltration into the periodontium. It has been reported that ER stress modifies the gene expression associated with transcription of proinflammatory cytokines in B cells. In addition, ER stress could be one of the pathological mechanisms in periodontal disease, evidenced by the colocalisation of HSP60/HSP70 cells and B cells in gingival biopsies from periodontitis patients.

Indeed, inflammatory cytokines can trigger UPR in periodontal tissues. The long-term rather than short-term inflammatory stimulation by tumour necrosis factor alpha (TNF-α) or interleukin-1β (IL-1β) could activate the prolonged UPR. ER stress is also detected in a hypoxia microenvironment accompanied by inflammatory infiltration in aberrant vascularisation in periodontal tissues.

Conversely, ER stress can manipulate the release of proinflammatory cytokines with multiple proinflammatory signalling pathways including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Salubrinal (elF2α phosphorylation modulator/ERS antagonist) treatment successfully repressed the secretion of proinflammatory cytokines IL-6 and IL-8 in hPDLCs induced by LPS. Consistently, IL-8 expression was elevated by thapsigargin, a selective inhibitor of the sarco/endoplasmic reticulum calcium ATPase for ER stress activation. Moreover, ER stress is also attributed to intracellular calcium overload and increased oxidative stress in periodontal inflammatory conditions.

**ER dysfunction in periodontal destruction**

Periodontal destruction occurs owing to dysbiosis of the host to external stimuli. The initial gingivitis will develop into severe chronic inflammation, and result in degradation of ligament fibres by MMPs and alveolar bone resorption by osteoclasts. When the ER fails to recover, the sustained ER dysfunction may lead to irreversible damage to the periodontal tissue. Uncovering the mechanisms of ER in periodontium destruction will facilitate the application of precision treatment to periodontal tissue regeneration.

**ER dysregulation in the gingiva**

ER stress induces autophagy and cell apoptosis via e-Jun activated protein kinase/stress-activated protein kinase (JNK/SAPK or p38 MAPK) in human gingival cells. Human gingival fibroblasts (HGFs) are essential for oral wound healing. Functional impairment to the HGFs and damage to the gingiva in the acidic intraoral environment are attributed to the excessive Ca2+ released from ER mediated by OGR1/phospholipase C signalling. Zoledronic acid (ZA), a bisphosphonate, can affect the viability of HGFs with irregularly shaped nuclei, dilated rough ER and numerous vacuoles. Cyclosporine can significantly augment ER stress and induce overgrowth of human gingiva via the prominent anti-apoptotic action. 4-phenylbutyric acid (4-PBA) attenuates the gingival hyperplasia by suppressing both the fibrotic and vascular components by blocking ER stress and matrix protein markers including TGF-α, connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) in HGFs.

**ER and ECM degradation**

MMP-mediated ECM degradation plays an important role in periodontal destruction. The ER triggers expression of MMPs by activating CCAAT enhancer binding protein beta (C/EBP β) expression in hPDLCs. Inhibition of ER stress by salubrinal and C/EBP homologous protein (CHOP)/GADD153 small interfering RNA (siRNA) eliminates ECM degradation and MMP pro-
duction in nicotine-treated PDLCs. Furthermore, ER stress contributes to PDLC apoptosis and periodontal connective tissue destruction via the Akt, ERK, p38, JNK, MAPK and NF-κB pathways in PDLCs. As a result, ER stress seems to play a regulatory role in PDLC apoptosis and ECM degradation in PDL destruction.

Role of ER in alveolar bone resorption
Alveolar bone homeostasis is sustained by the balance between osteoblastogenesis and osteoclastogenesis. An overwhelming amount of bone resorption over formation contributes to alveolar bone loss in periodontal disease. Alveolar bone loss is mainly modulated by the function of osteoblasts, osteoclasts and osteocytes. It has been reported that the ER stress activator thapsigargin or tunicamycin contributes to alveolar bone loss in experimental periodontitis mice. Furthermore, ER-associated pathways and activities have been shown to participate in modulating the function of these bone cells, suggesting that ER plays a crucial role in alveolar bone homeostasis. Aberrant osteocyte activity can lead to bone loss. General communication takes place between osteocytes through their dendritic network. Interestingly, osteocytes can share their mitochondria transferred by the ER from the donor to recipient cells. The degree of ER stress has been found to affect the viability and osteogenic differentiation of osteoblasts. ER stress can weaken the osteogenesis of primary calvarial osteoblasts (POBs). Additionally, salubrinal can restore the ER stress-induced decreased viability of POBs in a dose-dependent manner. It has been reported that the PERK-eIF2α and IRE1α–XBP1 pathways also participate in osteoclastic differentiation of bone marrow macrophages.

The ER is the largest organelle for intracellular Ca²⁺ storage. Calcium oscillations, which can be modulated by the calcium receptors on the ER membrane, are well-recognised in reactive oxygen species and receptor activator of NF-κB ligand (RANKL)-induced osteoclastogenesis and bone resorption via calcineurin. The production of RANKL in osteoblasts and PDLCs has been detected in periodontitis with the occurrence of alveolar bone damage.

ER mechanisms of periodontal regeneration
The ultimate goal of periodontal therapy is to regenerate the lost tissues. Notably, irreversible alveolar bone absorption as an outcome of periodontal disease is mainly attributed to the destruction of stemness of PDSLCs. The effectiveness of periodontal treatment based on PDLSCs has been widely supported in recent studies, as reported in a review. PDLSCs have the ability to differentiate into osteoblasts, cementoblast-like cells and collagen-forming cells, and thus can regenerate the periodontal tissues destroyed by chronic periodontitis. Given that ER dysfunction was found to be involved in the onset and progress of periodontal disease, treatments targeting the recovery of ER homeostasis in PDSLCs may present a novel strategy for the reconstruction of periodontal tissue.

ER stress inhibitors such as 4-PBA can directly recover the alveolar bone level in periodontitis models with their functions by improving the differentiation ability of PDLSCs. Low-intensity pulsed ultrasound (LIPUS) enhances the osteogenic ability of PDLSCs and rescues the alveolar bone loss in periodontitis mice by downregulating ER stress, which makes it a promising noninvasive strategy to reconstruct the periodontal tissues. Given the essential role played by PERK in the differentiation of hPDLSCs into osteoblast-like cells, the PERK inhibitor GSK2606414 can facilitate the formation of alveolar bone. CirkCDK8 silencing rescues impaired PDLSC osteogenesis under hypoxic conditions by alleviating the ER-associated autophagy, which indicates a novel therapeutic strategy to manipulate intracellular dynamics via the ER pathway.

Conclusion
The periodontium plays a crucial role in maintaining dental health. The main aim of periodontology is to rehabilitate the periodontal tissues. In recent years, ER has emerged as a regulator of cell function and trigger of periodontal disorders. Accordingly, ER-targeting therapy is increasingly harnessed to support regenerative therapeutics. With the help of technological advances, research focused on ER intracellular regulation and manipulating ER dynamics hold the prospect of safeguarding periodontal homeostasis and regeneration.

Conflicts of interest
The authors declare no conflicts of interest related to this study.

Author contribution
This review article was conceived by Profs Yan JIN and Fang JIN; Drs Qi Ming ZHAI and Bei LI were responsible for the preparation and revision of the initial
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