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Inflammaging: Molecular Pathways and Implications in Oral Pathology

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Inflammaging is known to be an aging-associated chronic, low-grade inflammatory state that significantly influences the pathophysiology of various age-related diseases, including those affecting oral health. This review explores the molecular and cellular mechanisms of inflammaging and its implications in oral pathology. We will review key factors contributing to inflammaging, including cellular senescence, and immune dysregulation, all of which induce pro-inflammatory cytokines and reactive oxygen species (ROS). These inflammatory mediators affect oral tissues, predisposing individuals to chronic conditions such as periodontitis, and dental pulp inflammation. Additionally, we will briefly discuss how the oral microbiome is involved in the regulation of inflammaging. Understanding the molecular pathways of inflammaging may provide valuable insights not only into oral health but also into potential health strategies for the aging population. [J Korean Dent Sci. 2024;17(4):174-86]

Key Words: Inflammaging; Oral diseases; Inflammation; Aging; Microbiome; Oral microbiome

Introduction

Aging is accompanied by a complex physiological changes that collectively contribute to increased vulnerability to age-related diseases. Among these changes, the concept of inflammaging has emerged as a prominent hallmark of the aging process, characterized by chronic low-grade inflammation that persists over time[1,2]. The term of inflammaging is referred to age associated low grade and chronic sterile inflammation with immune dysfunction. By the aging, divers cellular populations undergo senescence and accumulate in the organs. Cellular senescence is caused by inherent effect, such as telomere shortening, chromosomal instability,

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metabolic stresses, etc., and exogenous effects, such as chemical exposure, radiation, and environmental stresses. Senescence cells increase their gene expression encoding secreted protein. These changing of cellular phenotype is called senescence associated secretory phenotype (SASP). This process is associated with various age-related diseases and conditions, including cardiovascular disease, neurodegenerative diseases, diabetes, and even oral diseases. Inflammaging is driven by a complex interplay of cellular and molecular mechanisms. And the inflammaging contribute to oral disease progression. In this paper, we will briefly review the cellular and molecular mechanisms underlying the inflammaging and its implications on oral pathology. Moreover, we will briefly explore the relation between oral microbiome and the inflammaging.

Molecular and cellular mechanisms of inflammaging

Cellular mechanisms of inflammaging

At the cellular level, alterations in the immune system play a central role in perpetuating inflammation throughout the aging process. It is called immuno-senescence which has highly correlation with inflammaging (Fig. 1A). The innate immune system is affected by aging through functional deficits. Innate immune cells such as dendritic cells (DCs) and macrophage have role in chemotaxis, phagocytosis, antigen presentation, and the killing the bacteria or dead cells. However, aged DCs and macrophages show less response to signal from surface immune receptors, such as Toll-like receptors (TLR)[3-5]. Because skin and mucosal layer of aged tissue weak and venerable to outside intruders (ex-bacteria, and dead cell debris)[6], the DCs and macrophages are continuously exposed to pathogen with failing to clearance and remodeling the tissue, which eventually lead to chronic inflammation[3,7]. Moreover, aging promotes decline of adaptive immune system. This decline affects both arms of the adaptive immune system: T and B lymphocyte. Shrinkage of thymus, which is critical for T lymphocytes maturation, is characteristic of aging[8-10]. This reduces the output of naive T lymphocytes, leading to a smaller repertoire of T lymphocytes capable of recognizing new antigens[11]. Senescent T lymphocytes accumulate and exhibit impaired proliferation, cytokine production, and cytotoxic activity[12]. Especially, accumulation of senescent memory $CD8^+$ T lymphocytes and impaired regulatory T lymphocytes makes the host susceptible to novel pathogen and chronic inflammation[13,14]. Similarly, aged bone marrow cells show less stemness and differentiate into B lymphocyte. It makes reduction of naïve B lymphocytes and accumulates senescent B lymphocytes[8]. The senescent B lymphocytes produce less diverse repertoire of antibody with low affinity to antigen[15,16]. Many research paper explored auto-antibody produced from senescent B lymphocytes, which have auto-response to host antigen, which indicates possibility of autoimmune disease in aging[17-19].

Dysregulation of immune responses from both immune and non-immune cells contributes to the sustained activation of immune cells, characterized by a shift towards a pro-inflammatory state and a decline in anti-inflammatory mechanisms. Senescent cells are key mediators of inflammaging, which accumulate with age and exhibit a pro-inflammatory phenotype known as SASP[1,20,21] (Fig. 1B). The SASP is a unique secretory profile characterized by the secretion of a myriad of factors, including pro-inflammatory cytokines, chemokines, growth factors, proteases, and extracellular matrix remodeling enzymes[20]. The composition of the SASP can vary depending on the cell type, senescence-inducing stimulus, and microenvironmental context. Common components of the SASP include interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), matrix metalloproteinases (MMPs), and chemokines such as monocyte chemoattractant protein-1 (MCP-1). These secreted factors create a local microenvironment that promotes inflammation, tissue remodeling, and immune cell recruitment[22]. The SASP

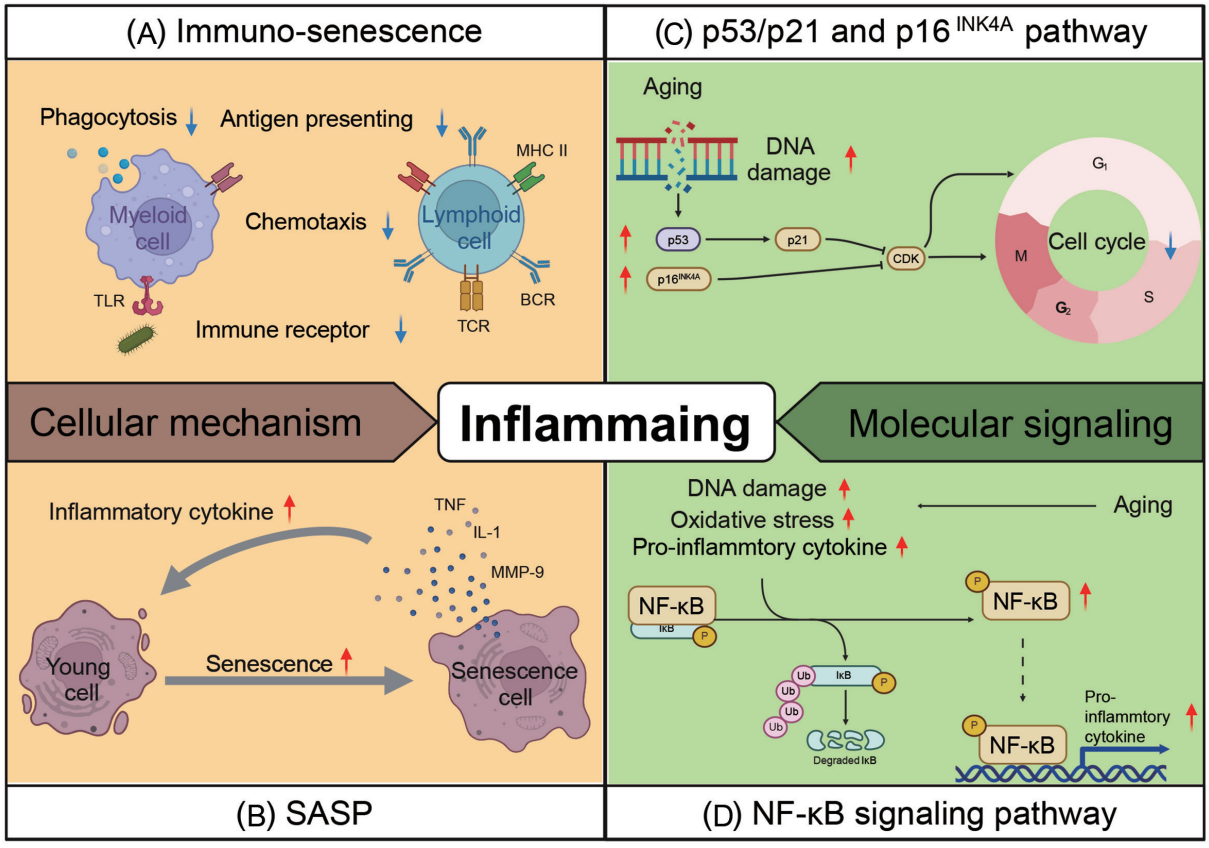

Fig. 1. Cellular mechanisms and Molecular signaling pathways of inflammaging in senescence cells. Senescence immune cells have less ability of phagocytosis, immune receptor expressions, chemotaxis, and antigen presenting (A). Senescence cells show unique characteristics, called senescence-associated secretory phenotype (SASP). They continuously secrete low graded pro-inflammatory cytokines. The secreted cytokines can affect nearby cells to be exposed chronic inflammation (B). The DNA damages in senescence cells activate p53, p21, and $p16^{INK4A}$, which inhibit the cyclin-dependent kinase (CDK). As a result, cell cycle in senescence cells is arrested in G1 phase (C). Consistent exposing to pro-inflammatory cytokines, oxidative stress, and DNA damages can activate NF-κB signaling pathway. NF-κB is key transcription factor for pro-inflammatory cytokines (D). As a result, complex connections between cellular mechanism and molecular signaling pathway in senescence cells can lead aged tissue fail to regeneration and maintain homeostasis. BCR: B-cell receptor; IL-1: Interleukin-1; IκB: NF-kappa B inhibitor; NF-κB: Nuclear factor-kappa B; MHC II : Major histocompatibility complex class II ; MMP-9: Matrix metalloproteinase-9; $p16^{INK4A}$: Cyclin-dependent kinase inhibitor 2A; p21: Cyclin-dependent kinase inhibitor 1; p53: Cellular tumor antigen p53; TCR: T-cell receptor; TLR: Toll-like receptor.

can also have systemic effects, as senescent cells can disseminate pro-inflammatory signals to distant tissues via circulation. In healthy condition, the SASP can have beneficial effects by promoting immune surveillance and tissue repair in response to stress or injury[23]. However, chronic exposure to SASP components can drive persistent inflammation, tissue dysfunction, and contribute to the pathogenesis of age-related diseases, including cancer, cardiovascular disease, neurodegenerative diseases, and metabolic disorders[24].

Molecular pathways in inflammaging

In addition to immune dysregulation, alterations in signaling pathways contribute to the development

and maintenance of inflammaging[25]. Because accumulation of senescent cells accompanies inflammaging[23], understanding the signaling pathway for cellular senescence is important. In aging, persistent activation of this pathway due to chronic DNA damage or oxidative stress leads to a permanent cell cycle arrest and contributes to the accumulation of senescent cells. At that time, key signaling pathways involve in cellular senescence, such as the p53/p21, $p16^{INK4a}$, and nuclear factor-kappa B (NF-κB) pathways and become dysregulated with age, leading to increased cellular senescence and SASP activation[26,27].

p53, tumor suppressor protein, becomes activated and transcriptionally upregulates the expression of various target genes involved in cell cycle arrest, DNA repair, apoptosis, and senescence in response to stress signals, such as DNA damage within the aging[28]. One of targets of p53 is p21 (also known as cyclin-dependent kinase inhibitor 1A, CDKN1A), a potent inhibitor of cyclin-dependent kinases (CDKs) that regulates cell cycle progression[29,30]. Activation of the p53/p21 pathway results in the inhibition of CDK activity and subsequent arrest of the cell cycle at the G1 phase, preventing damaged cells from proliferating and perpetuating genomic instability[31]. This growth arrest allows cells to undergo DNA repair or undergo apoptosis if the damage is irreparable. The $p16^{INK4a}$ tumor suppressor gene encodes for the p16 protein, a cyclin-dependent kinase inhibitor that specifically inhibits the activity of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6). These kinases normally phosphorylate and inactivate the retinoblastoma protein (Rb), allowing progression through the G1 phase of the cell cycle. By inhibiting CDK4/6 activity, $p16^{INK4a}$ prevents Rb phosphorylation and promotes cell cycle arrest at the G1 phase[27,32]. Activation of the $p16^{INK4a}$ pathway leads to cell cycle arrest and senescence in response to various stress signals, including reactive oxygen species (ROS), oncogene activation and telomere dysfunction[33-35]. The accumulation of $p16^{INK4a}$ positive senescent cells has been implicated in aging and age-related diseases, as these cells contribute to tissue dysfunction and inflammation through the secretion of the SASP[36] (Fig. 1C).

In the context of cellular senescence, NF-κB signaling is intricately linked to the establishment and maintenance of the senescent phenotype, as well as SASP. NF-κB signaling can be activated in response to various stress stimuli, including DNA damage, oxidative stress, and pro-inflammatory cytokines[27]. Once activated, NF-κB translocate from the cytoplasm to the nucleus, where it binds to specific DNA sequences known as κB sites and regulates the expression of target genes involved in inflammation, immune responses, and senescence. NF-κB induces the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, such as CCL2, IL-6, IL-8, MCP-1, and intercellular adhesion molecule-1 (ICAM-1), which constitute components of the SASP[37,38]. These signaling pathways converge to promote a state of chronic inflammation in senescent cells that contributes to tissue damage, functional decline, and the pathogenesis of age-related disease (Fig. 1D).

Inflammaging in the oral health

The oral cavity is essential for nutrition, communication, and social interaction, but it becomes increasingly vulnerable to inflammation with age. These conditions often bring pain, discomfort, and altered appearance, profoundly impacting quality of life. Inflammaging plays a key role in these conditions by weakening gums, reducing bone support, and impairing saliva's protective functions, creating a favorable environment for infections. Similarly, aging-related changes in saliva, which is a natural defense mechanism, reduce its ability to wash away harmful bacteria, further tipping the scales in favor of disease[21,39-41] (Fig. 2). In this part we will briefly review several oral diseases that are related to inflammaging.

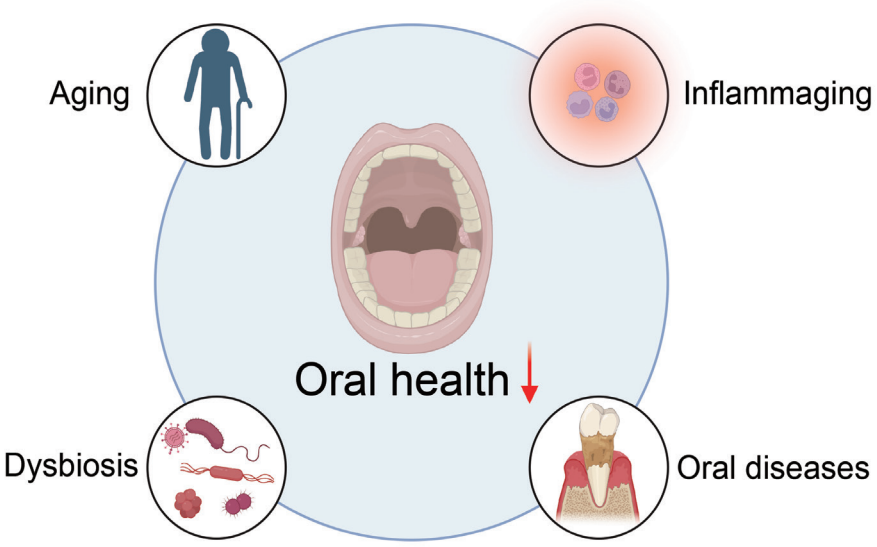

Fig. 2. The multiple relationships for oral health. Aging is one of the most important factors for maintaining oral health. By aging, accumulation of senescent cells can induce mild and chronic inflammation (inflammaging). And oral microbiome can be altered to harmful bacterial communities (dysbiosis). These changes can make oral conditions vulnerable to several oral diseases.

Periodontitis

Periodontitis is a chronic inflammation in periodontium which encompass with consistent damage to cementum, periodontal ligament, and alveolar bone[42]. While the exact mechanisms underlying this inflammation remain unclear, bacterial infection, particularly from *Porphyromonas gingivalis*, is considered a primary etiological factor[43]. Emerging evidences suggest a strong correlation between periodontitis and inflammaging, as chronic inflammation in periodontitis has been shown to induce cellular senescence in periodontal tissues. For example, a recent study using human gingival tissue showed that cellular senescence p16 mRNA level in the tissue is increased in periodontitis compared to healthy group with recession of tissue. And the magnitude of incensement of $p16^{INK4A}$ level was much higher in old group (>66.2 y) than young group (<41.67 y). Additionally, the pro-inflammatory cytokine IL-8 mRNA levels were higher in healthy gingival tissue from older individuals compared to younger individuals, suggesting an age-dependent inflammatory baseline[44]. Further supporting these findings, repeated exposure to lipopolysaccharide (LPS) from *Porphyromonas gingivalis* in primary alveolar osteocytes induced cellular senescence, marked by increased expression of $p16^{INK4A}$, p21, and p53. And repeatedly LPS treated osteocyte robust expressed IL-1α, IL-6 and TNFα[45]. These indicate that chronic inflammation can induce senescence to periodontium cells with inflammaging.

Conversely, inflammaging appears to exacerbate periodontal tissue dysfunction through SASP activity. With the aging, the gingival fibroblast gradually undergoes functional changes. The gingival fibroblasts located in lamina propria produce collagen to maintain the tissue strength and elasticity. In the case of aging, senescence gingival fibroblasts show upregulation of SASP. When human gingival fibroblasts (HGFs) were exposed to oxidative stress (e.g., hydrogen peroxide exposure), HGFs expressed aging related cell cycle protein such as p53, $p16^{INK4A}$, and p21 accompanied with expressing SASP factors such as IL-6,8, 17, TNFα, and IL-1β[46]. Similarly, inflamed gingival tissues showed elevated levels of senescence markers, ROS, and cyto-

kines such as IL-1β, IL-6, TGF-β, and IL-8. Notably, senescent HGFs promoted macrophage polarization to the pro-inflammatory M1 phenotype ($CD86^{+}$) when cultured in vitro, demonstrating the potential of senescent fibroblasts to perpetuate chronic inflammation[47].

The pro-inflammatory cytokines produced during inflammaging recruit and activate immune cells, including macrophages, neutrophils, and lymphocytes. While controlled immune responses are essential for host defense, chronic and dysregulated immune activation can result in damages to periodontal tissues[48,49]. The chronic immune response associated with periodontitis also impacts alveolar bone, a defining feature of the disease. Pro-inflammatory cytokines, particularly receptor activator of nuclear factor kappa-B ligand (RANKL), stimulate osteoclast activity, leading to increased bone resorption. Concurrently, the presence of senescent osteoblasts with diminished reparative capacity exacerbates the imbalance between bone formation and resorption[50-52]. As this cycle continues, alveolar bone loss progresses, ultimately resulting in tooth mobility and loss. Furthermore, SASP factors released by senescent periodontal cells, such as IL-8 and MMP-9, perpetuate connective tissue degradation, compounding the structural damage[53,54].

Together, these findings highlight the complexity of periodontitis as a disease that is not merely driven by bacterial infection but also by the interplay of chronic inflammation, cellular senescence, and inflammaging (Fig. 3). This intricate network of processes creates a

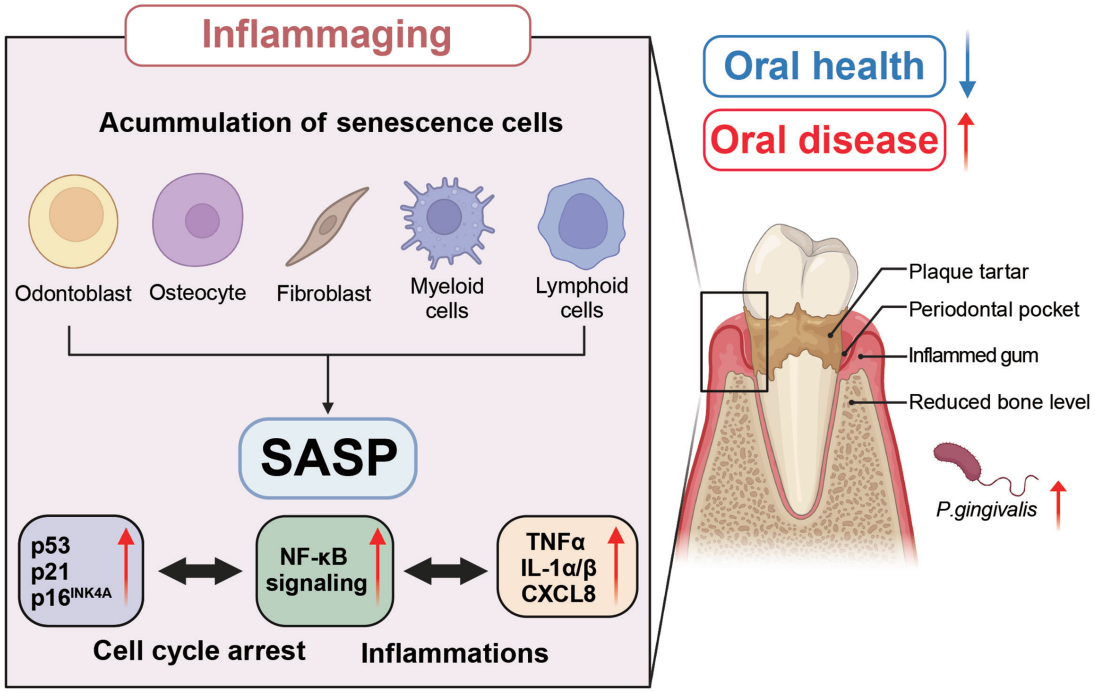

Fig. 3. The impact of inflammaging on oral health. The inflammaging is one of the most important factors in balance between oral health and diseases, such as periodontitis and pulpitis. Oral diseases can induce cells in periodontium to cellular senescence with exhibiting SASP. These cells show cell cycle arrest with p53, p21, and $p16^{INK4A}$ activation and inflammation with NF-κB signaling pathway. Conversely, accumulation of senescence cells in periodontium make tissue venerable to oral disease. These events consequently make the oral tissue weak and easy to getting damage under oral microbacterial dysbiosis condition. CXCL8: Chemokine (C-X-C motif) ligand 8; IL-1α: Interleukin-1 alpha; IL-1β: Interleukin-1 beta; NF-κB: Nuclear factor-kappa B; $p16^{INK4A}$: Cyclin-dependent kinase inhibitor 2A; p21: Cyclin-dependent kinase inhibitor 1; p53: Cellular tumor antigen p53; TNFα: Tumor necrosis factor alpha.

self-perpetuating cycle of tissue damage and immune dysregulation. Addressing periodontitis effectively requires a approach that targets not only bacterial pathogens but also the inflammatory and senescence pathways that contribute to its progression.

Dental pulp inflammation

Dental pulp is the soft tissue located at the center of a tooth, surrounded by dentin and cementum. It consists of the pulp chamber in the crown, root canals in the roots, and the apical foramen at the root tip, which allows blood vessels and nerves to enter. This structure supports tooth growth, sensation, and repair[55,56]. Dental pulp inflammation, so called pulpitis, is the inflammation of the dental pulp. It occurs when the pulp is irritated or damaged, often due to bacterial infection, trauma, or deep dental decay[56-58].

Moreover, the relationship between pulpitis and inflammaging is a growing area of interest in dental and systemic health research. While direct studies specifically linking pulpitis to inflammaging are limited, emerging evidence suggests a possible correlation based on shared inflammatory mechanisms and molecular pathways. When the bacteria invade the dental pulp after dental decay, odontoblasts first encounter the invasion because they locate beneath dentin of crown part. They naturally express TLR and nucleotide binding and oligomerization domain like receptors[59,60]. After the odontoblasts recognize the bacterial infection through the receptors, they secrete beta-defensin (BD) which is cationic host defense peptides[61]. BD is known to not only inhibit microorganisms' viability, but also induce host cell's immune response, such as pro-inflammatory cytokines and chemokines production[62-64]. At the same time, pulpal dendritic cells (pDCs) also recognize pathogens and accumulate in boundary between dentin and dental pulp during surveillance of the tissue[65,66]. They process bacterial peptides and present them through major histocompatibility complex (MHC) to induce adaptive immune cell activation[67]. And then pulpal fibroblasts recognize pathogen and also release pro-inflammatory cytokines and chemokines[68]. As a result, innate immune cells (macrophage, pDC, etc.) and adaptive immune cells (B lymphocyte, and T lymphocyte) can be activated and accumulated in damaged areas.

Numerous studies have identified the immune mechanisms of pulpitis. For example, when the odontoblast-like cells and pulpal fibroblast were exposed to lipoteichoic acid (ligand for TLR 2), TNFα and CXCL8 gene expressions were dramatically increased within a few hours. Also, immature DCs highly secreted TNFα, CXCL8, and IL-1β[68]. Using cDNA arrays, another study showed that not only proinflammatory cytokines (IL-1β, TNFα, and Lymphotoxin α) but also chemokines (CCR2, CCR4, CCR5, CCR9, CCL3, CCL12 and CCL23) genes were significantly increased in odontoblast layer and pulp layer from human carious teeth compared to healthy[69]. A recent study confirmed that numerous immune cells, such as naïve B cells, plasm cells, $CD8^+$ T cells, M0 macrophages, M2 macrophages, dendritic cells and neutrophils, infiltrated into pulp tissue in pulpitis patients, based on gene expression omnibus dataset analysis. And their biological functions were up-regulated in immune cell chemotaxis, cytokine-mediated signaling pathway, and immune cell migration[70].

This inflammation related cellular mechanisms are shared in aged pulp tissue. Senescence dental pulp cells exhibit reduced regenerative capacity and an altered secretory profile into SASP that includes increased production of pro-inflammatory cytokines such as IL-6 and IL-8. The shift contributes to the sustained inflammatory state within the dental pulp, exacerbating tissue degradation and impairing repair processes. Because inflammatory cytokines can affect nearby cells making it hard to differentiate, and regeneration, which contributes to aggravation dental pulp[71]. Moreover, inflammaging influences dental pulp cells-mediated immune responses by modulating signaling pathways, such as NF-κB and MAPK, which are critical in cytokine production. These alterations can lead to

a compromised ability to respond effectively to infections or injuries, contributing to a higher susceptibility to pulpitis and other age-related dental diseases[53] (Fig. 3).

Inflammaging and oral microbiome

The oral microbiome plays a crucial role in human health, significantly impacting systemic immune responses and inflammatory processes. There is growing evidence linking inflammaging to the dysregulation of the oral microbiome. Healthy individuals typically exhibit a balanced oral microbiota that maintains stable host-microbial interactions. This homeostasis regulates immune responses and prevents the over-activation of inflammation. Aging is a factor associated with, or even inducing, a reduction in microbial diversity within the oral environment, along with an increase in pathogenic microbiota, ultimately leading to immune dysregulation.

Elderly people often experience periodontitis, dental caries, and oral candidiasis. These conditions can contribute to systemic inflammation, potentially exacerbating chronic diseases such as diabetes, rheumatoid arthritis, and cardiovascular disease. Periodontitis, in particular, has been linked to elevated levels of systemic inflammatory markers, which may increase the risk of cardiovascular events[72]. Therefore, maintaining oral microbiome homeostasis in elderly adults is crucial for reducing the risk of systemic inflammatory diseases.

Multi-geographical population studies have documented that aging involves several changes in the composition and function of the body's microbiome homeostasis. Results suggest that aging is associated with a decrease in anti-inflammatory bacterial species, including *Faecalibacterium* and *Roseburia*[73,74]. In addition to the reduction in anti-inflammatory bacteria, aging impacts bacterial biodiversity and increases the proportion of potentially harmful bacterial families, such as *Streptococcaceae* and *Staphylococcaceae*[73-75]. The oral cavity microbiota is highly diverse, with more than 700 species identified to date. Among these, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and Spirochaetes are reported as the dominant phyla[76-78]. Limited information is available on the relationship between the oral microbiota and aging. A study analyzed the oral microbiome of elderly individuals residing in nursing homes and compared it with the oral microbiome of those living independently[79]. They found that nursing home-residing group showed less diverse microbiome phyla compared with elderly individuals living independently[79]. Microbiome analysis showed phyla such as *Actinomyces*, *Streptococcus*, *Bacilli*, *Selenomonas*, *Veillonella*, and *Haemophilus* were abundant in nursing home-residing group. However, *Prevotella*, *Leptotrichia*, *Campylobacter*, and *Fusobacterium* were relatively lower in nursing home-residing group compared with elderly individuals living independently[80].

It is known that female hormones play important roles in maintaining microbiota homeostasis and influence oral health. In particular, Estrogen is involved in the regulation of the oral mucosa and salivary glands. Estrogen receptors have been identified in the oral cavity and gingiva, suggesting their role in regulatory processes[81]. Estrogen deficiency also impacts on salivary flow and postmenopausal women showed significant lower[82]. In addition to alterations in host environment during menopause, oral microbiome composition and functions are also affected significantly. Studies showed that the changes of hormone by aging in women may induce favor environment for the growth of periodontal pathogens including *Porphyromonas gingivalis* and *Tannerella forsythia*[83,84]. Together with human data, in vivo animal study using an ovariectomized rodent (rat) also found that the estrogen shortage dysregulates oral microbiome homeostasis[85], suggesting that the aging and hormonal changes by aging may impact significantly on the oral microbiota.

Conclusion

Aging is a complex process characterized by physiological changes that profoundly affect both systemic and oral health. Inflammaging, a chronic, low-grade inflammatory state driven by immune dysregulation, cellular senescence, and alterations in signaling pathways, is a hallmark of aging. It is closely associated with age-related oral diseases and the decline of systemic body functions.

This manuscript explores the interplay between inflammaging, aging, and the oral microbiome, emphasizing the need for further research to elucidate the underlying mechanisms linking inflammaging to systemic and age-related diseases. Developing strategies to mitigate inflammaging or create targeted interventions could play a crucial role in maintaining overall health and preventing age-associated conditions.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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