Concise review

Emerging Roles of Periodontal Pathogen–Derived Outer Membrane Vesicles in NAFLD

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ABSTRACT

The rising incidence of nonalcoholic fatty liver disease (NAFLD) poses a great socioeconomic burden worldwide. Also, periodontitis is the most common chronic inflammatory disease caused by a group of oral pathogens, affecting both oral health and systemic conditions, especially liver disease. Although accumulating evidence has elucidated an association between periodontal pathogens and NAFLD, the role of periodontal pathogen–derived outer membrane vesicles (OMVs) has not yet been clarified. In this comprehensive review, we aim to address this gap by summarising the progression and pathogenesis of NAFLD and revealing the relationship between periodontal disease and NAFLD multidimensionally. Additionally, this review sheds light on the multifunctional roles of periodontal pathogens OMVs and emphasises that periodontal pathogen–derived OMVs promote the development of NAFLD by stimulating Kupffer cells to produce inflammatory factors and inducing the activation of Hepatic stellate cells. However, it is still controversial whether periodontal pathogen–derived OMVs can be transferred to the liver through the bloodstream route or the oral-gut-liver axis. This highlights the pressing need for continued research efforts to develop new and optimised research schemes to observe the formation of the systemic distribution pathway of periodontal pathogen–derived OMVs. Finally, it is notable that there are currently no relevant clinical treatment guidelines to make specific provisions on controlling the level of periodontal pathogen–derived OMVs in patients with NAFLD. Guidelines developed based on our findings may contribute to the standardisation of practices. It can also provide effective strategies and potential therapeutic targets for NAFLD patients with periodontitis to alleviate the development of NAFLD diseases by inhibiting periodontal pathogens OMVs.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as a spectrum of liver disorders, varying in severity from simple fat accumulation referred to as hepatic steatosis or nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) manifested by lobular inflammation and hepatocellular damage, advanced fibrosis and eventually cirrhosis.[1] At the stage of cirrhosis, there is extensive scaring, and the liver function of patients is irreversibly impaired with a high risk of developing hepatocellular carcinoma (HCC), which can be life-threatening without liver transplantation.[2] What's worse, the majority of patients with NAFLD are asymptomatic and may

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remain unaware until it has progressed into cirrhosis.[3] Unlike other highly prevalent diseases, NAFLD has received less attention from the global public health community.[4] In fact, NAFLD is one of the most frequent chronic liver diseases worldwide, with an overall global prevalence of 30%, varying from 44.37% in Latin America to 25.1% in Western Europe.[5] Moreover, the prevalence has been on the rise over the past three decades, posing a significant social and economic burden on global health.[4] Although the cause and pathogenic processes of NAFLD are not clearly understood, it has been reported to be associated with environmental factors, metabolic obesity, congenital and acquired lipodystrophy, genetic causes, endocrine disorders and drugs, among other factors.[6] Recently, accumulating evidence has supported an association between NAFLD and periodontal disease.[7-11]

Periodontal disease is the most common chronic inflammatory and infectious disease caused by a group of periodontal pathogens.[12] These pathogens, such as *P. gingivalis, Tannerella forsythia, Treponema denticola* and *Actinobacteria*, harbour a variety of virulence factors, leading to evasion of natural defences and destruction of tissue surrounding the tooth, specifically gingiva, periodontal ligament, alveolar bone and cementum.[13] Periodontitis is an advanced condition of periodontal disease characterised by gingival inflammation and loss of connective tissue attachment. The prevalence of periodontitis is about 47% in adults in the United States.[14] It can not only cause oral health conditions, such as tooth mobility and even tooth loss, but also affect systemic diseases (Figure S1). According to the most recent study, periodontitis is mainly related to nutritional and cardiovascular diseases.[15] Epidemiological studies have found that the levels inflammatory mediators (C-reactive protein and prothrombotic factor) and dyslipidaemia related to atherosclerosis increased significantly in patients with periodontitis.[16] Moreover, periodontal disease has been shown to be independently related to kidney disease, lung disease (obstructive sleep apnoea, chronic obstructive pneumonia disease and COVID-19 complications),[17] rheumatoid arthritis,[18] viral infection and Alzheimer's disease.[19] Recent studies have found that the relationship between diabetes,[20] cardiovascular diseases[21] and periodontal diseases may be due to bacteremia,[22] oxidative stress[23] and increased release of nitric oxide caused by periodontal pathogens,[24] but the specific mechanism of nitric oxide in it is still unknown. Notably, there is a potential relationship between periodontal disease and NAFLD,[7] but a large number of samples and prospective design are still awaited to understand this problem better.

Recently, oral pathogenic bacterial OMVs have gained interests. OMVs are nanoscale vesicles spontaneously formed from the outer membrane of Gram-negative bacteria.[25] These vesicles are rich in various bacterial components including proteins, lipids, nucleic acids and pathogenic factors.[26] OMVs fulfil several roles in adhesion, invasion and damage to cells, as well as in bacteria-bacteria and bacteria-host cells interactions, modulating the host's immune response, biofilm formation and promotion of virulence.[27] Additionally, the most recent study indicated the potential link between oral pathobiont-derived OMVs and some systemic diseases.[28] However, a comprehensive review systematically addressing the relationship between oral pathogenic bacterial OMVs and chronic liver diseases, especially NAFLD, is lacking.

Therefore, in this review, we aim to bridge this gap by categorising the pathogenesis of NAFLD and the evidence of the relationship between periodontal disease and NAFLD. The opportunity was also taken to systematically evaluate the multifunctional roles of periodontal pathogens OMVs in NAFLD at the cellular level, to provide effective strategies and potential therapeutic targets for NAFLD patients with periodontitis.

Material and methods

Data sources

Two investigators (CCL and KKS) performed an electronic search in English using the Medline database via PubMed, Scopus, Embase, Web of Science, LILACS, Cochrane Library databases and Google Scholar. The search strategy focused on studies published in English up to February 2025 without any time restriction, using the following search terms: "NAFLD" OR "MAFLD" and "Periodontal disease" OR "Periodontal pathogens" and "OMVs" OR "EVs".

Primary objective

To systematically review the pathological progress of NAFLD and investigate the epidemiological evidence of the correlation between its incidence and periodontal disease.

Second objective

To systematically review and analyse the evidence of the long-distance transportation of periodontal pathogens OMVs to the liver and its multifunctional role in NAFLD. It is more important to find a new way to treat NAFLD by controlling the level of periodontal pathogens OMVs.

Study selection

Inclusion criteria

- Clear identification of the authors and the publication date
- Articles that have been published
- Studies that are publicly accessible

Exclusion criteria

- Opinion articles, descriptive studies and letters to the editor
- Studies on diseases not related to the periodontal pathogen–derived OMVs and NAFLD
- Studies lacking a clear control group or proper grouping
- Studies not written in English

The Progression and Pathophysiology of NAFLD

Traditionally, fatty liver disease that occurs independently of substantial alcohol intake is classified as NAFLD.[29] However, the term *metabolic dysfunction–associated fatty liver disease*

(MAFLD) has been recently proposed to encapsulate more precisely the metabolic abnormalities accompanying hepatic steatosis.[30,31] Despite this, the adoption of MAFLD as a classification has sparked debate, primarily because it does not exclude other aetiologies of liver pathology, including excessive alcohol use or viral hepatitis.[32] Thus, until there is a consensus in the scientific community, this article will use the term NAFLD.

NAFLD encompasses a spectrum of hepatic disorders. Hepatic steatosis, the initial phase of nonalcoholic fatty liver disease, is typically marked by an increase in hepatic triglyceride storage. This accumulation may result from *de novo* lipogenesis (DNL) in the liver, using fructose-derived fatty acids as a substrate or from the mobilisation of free fatty acids during the lipolysis of adipose tissues.[33]

NAFLD becomes primarily concerning when it advances to NASH, which is characterised by steatosis, liver injury, and inflammation. At this stage, cellular and immunological characteristics are pronounced. In the aspect of host cells, hepatocyte apoptosis occurs, which precipitates the release of proinflammatory mediators. Also, the hepatic sinusoidal endothelial cells contribute to the inflammatory milieu by producing cytokines and chemokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and C-C motif chemokine ligand 2 (CCL-2).[34] In terms of immune mechanisms, B cells play a critical role in the pathogenesis of NASH. Within the context of murine NASH models, B cells exhibited proinflammatory activity mediated by both B-cell receptor –dependent adaptive immune responses and MYD88-dependent innate immune pathways.[35] These cells engage in the production of immunoglobulins, antigen presentation and cytokine secretion. The activation occurs via pathogen-associated molecular patterns (PAMP) that are recognised by Toll-like receptors, thus contributing to immune-mediated inflammation through multiple pathways.[35,36]

Liver cirrhosis, a condition marked by end-stage damage to the liver, arises from acute or chronic exposure to pathogenic factors such as hepatitis, alcohol consumption, and obesity.[37] Prolonged inflammation leads to the replacement of normal liver parenchyma with fibrotic tissue and regenerative nodules, culminating in portal hypertension. This progression can further escalate into HCC, representing a clinically advanced stage that frequently necessitates liver transplantation as a therapeutic intervention (Figure 1).[38]

NAFLD does not invariably progress to NASH. The multiple-hit hypothesis considers that the progression from NAFLD to NASH requires additional metabolic insults, insulin

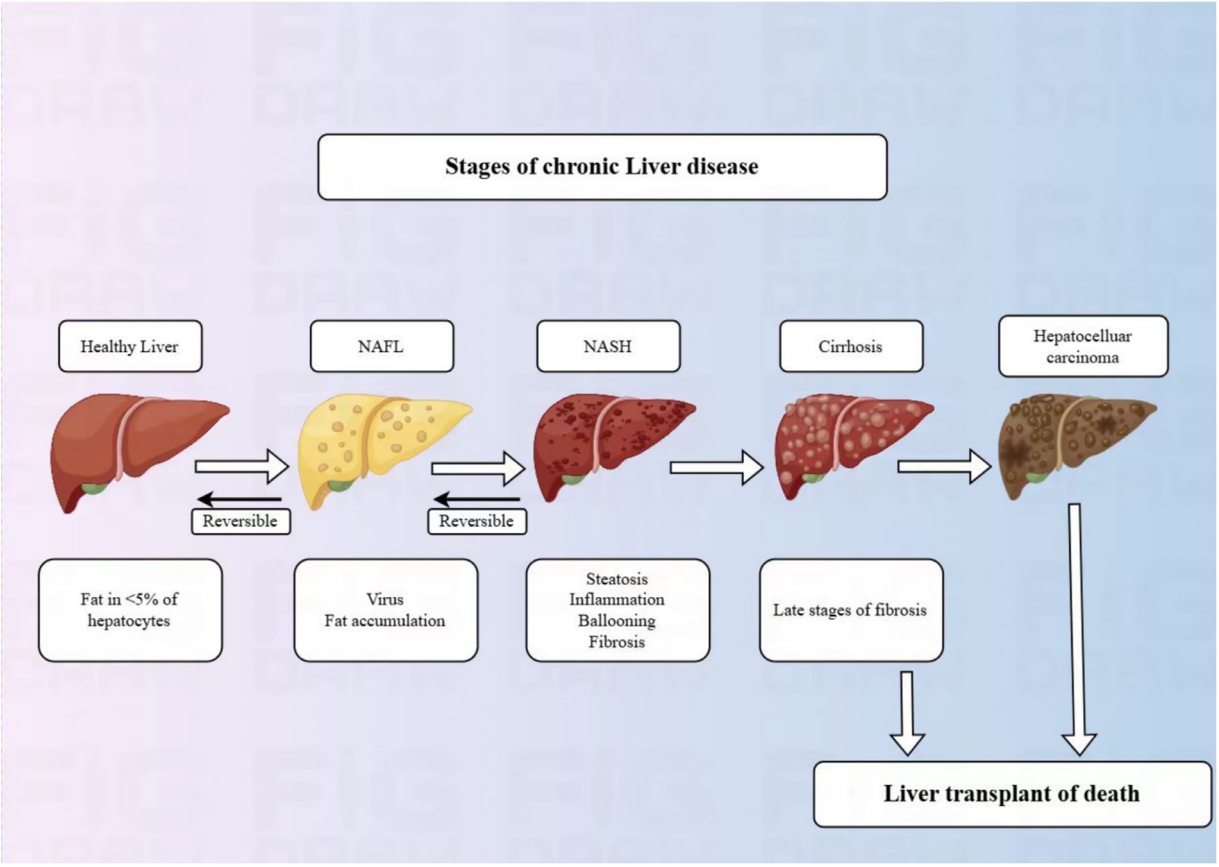

Fig. 1 – Progression of chronic liver diseases. This diagram illustrates the sequential stages of chronic liver conditions, starting from a healthy liver, advancing through nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH), cirrhosis and ultimately leading to hepatocellular carcinoma or the necessity for liver transplantation or death. The process highlights the reversibility of early stages and the dire consequences of disease progression.

resistance, oxidative stress, inflammation, nutritional factors, and other genetic and epigenetic factors.[39] These factors activate cellular stress pathways, culminating in hepatocyte apoptosis, inflammatory responses and the eventual development of fibrosis. Liver lipotoxicity exacerbated by insulin resistance has emerged as a crucial contributor to NAFLD pathogenesis. It has been reported that insulin acts as a potent enhancer of hepatic DNL chiefly by activating sterol regulatory element-binding protein (SREBP)-1c, which is instrumental in the transcriptional control of lipogenesis and initiates the genetic sequence required for fatty acid creation.[33] Additionally, insulin fosters the hepatic assimilation of free fatty acids in a dose-responsive manner, which in turn augments DNL.[40] The nexus between insulin resistance and hepatic lipotoxicity is apparent in the progression of NAFLD/NASH. In a state of insulin resistance, insulin perpetuates DNL while its capacity to suppress hepatic gluconeogenesis diminishes.[41]

Relationship Between Periodontal Disease and NAFLD

Although many studies have gradually revealed that periodontal disease is a risk factor for systemic diseases, the relationship between periodontal diseases and NAFLD has not yet been fully clarified.

Furuta et al.[42] included 2,459 first-year students at Okayama University in an early cross-sectional study conducted in 2010. They underwent compulsory oral examinations in April 2008, so this study did not go through the inclusion and exclusion process. Then, alanine aminotransferase (ALT) and probe bag depth (PPD) were detected in 2,225 students aged 18-19 years. Subjects with the presence of one or more teeth with PPD ≥ 4.0 mm were defined as having periodontitis. Three criteria for serum ALT were defined as normal (≤20 U/L), subclinical (21–40 U/L) and abnormal (≥41 U/L). It was found that the incidence of periodontitis was 5.8% in male subjects and 3.2% in female subjects. In logistic regression analysis, the serum ALT level of males with periodontitis was significantly higher than those without periodontitis. However, there is no significant relationship between the two in females.

Another research study on the association between periodontal disease and NAFLD was conducted in the United Kingdom in 2017. In this study, liver ultrasound and oral examination data of 8,172 participants were extracted and analysed statistically after excluding those who did not have a liver ultrasound examination (n = 814) or could not be graded by ultrasound examination (n = 127). The results indicated that hepatic steatosis was independently associated with periodontitis, and periodontal disease was more severe in advanced NAFLD patients.[43]

Using a subset of the data available in the National Health and Nutrition Examination Surveys (NHANES) from 2009 to 2012, Wiener et al.[44] included 5,758 eligible participants aged between 30 and 69 years with complete data on periodontitis and serum alanine aminotransferase. The relationship between them was investigated. According to the principles used by the Centers for Disease Control, and Prevention (CDC) and American Periodontology Socienty, periodontitis was classified into mild periodontitis, moderate periodontitis and severe periodontitis, and serum alanine aminotransferase was set at 40 IU/L as the critical value. Social demography and behavioural variables were also analysed as common factors. The percentages of periodontitis patients with serum alanine aminotransferase ≥40 IU/L and <40 IU/L were 38.2% and 39.2% respectively. Logistic regression analysis showed that the corrected odds ratio of alanine aminotransferase was 1.17, which was ≥40 IU/L. When periodontitis was the dependent variable, there was no statistical significance. Racial differences (comparison between Americans and Japanese) may lead to different results.

In a population-based cohort study by Akinkugbe et al.,[45] 6,265 adults aged between 20 and 79 years were invited from the eligible residents of West Pomerania in 1996 to participate. After excluding the participants who were missing initial data, drinking too much, missing liver ultrasound images, having edentulous jaws, suffering from chronic or autoimmune viral hepatitis and taking drugs to promote steatosis, a total of 2,481 individuals finally participated in this experiment. The levels of serum C-reactive protein (CPR) and a weighted genetic CPR score, representing the inflammatory burden, were then evaluated in this cohort. Periodontitis was categorised as the percentage of sites with PPD ≥4 mm (0%, <30%, ≥30%), and the NAFLD status was detected using ultrasound assessment. Logistic regression models determined that the prevalence of NAFLD was higher in participants with a high percentage of sites with PPD. Furthermore, periodontitis and NAFLD were associated with the level of serum CRP instead of weighted genetic CRP, but the corresponding estimate varied for participants with different serum CRP levels. This leads to a conclusion that periodontitis was correlated with higher prevalence odds of NAFLD, and serum CRP levels could modify the relationship positively.

In the study by Li Tan et al.,[46] participants over 40 years of age were included for whom the complete medical and socio-economic status information was collected, who received clinical periodontal examination, and who provided serum samples to evaluate IgG antibody titre against periodontal pathogens. Except for subjects for whom liver ultrasound examination data were lacking, the final experimental sample was 6,330 participants to explore the association between the incidence of NAFLD and high serum IgG antibodies for periodontal pathogens. The results showed that NAFLD risk factors are significantly related to the socio-economic status of the population, highlighting its multifactorial nature. Simply comparing the mean probing depth and the level of mixed antibody of *P. gingivalis* can show that the prevalence of NAFLD in patients with periodontal disease is significantly increased. However, after adjusting for confounding variables (such as age, sex, smoking status, and education level), these associations were no longer statistically significant.

In summary, most *in vivo* animal models, *in vitro* basic research and cross-sectional studies support the relationship between periodontal disease and NAFLD.[10] The common risks factors include age, gender, smoking, nutrition, race, medication, co-morbidities and genetics (Figure 2). However, this

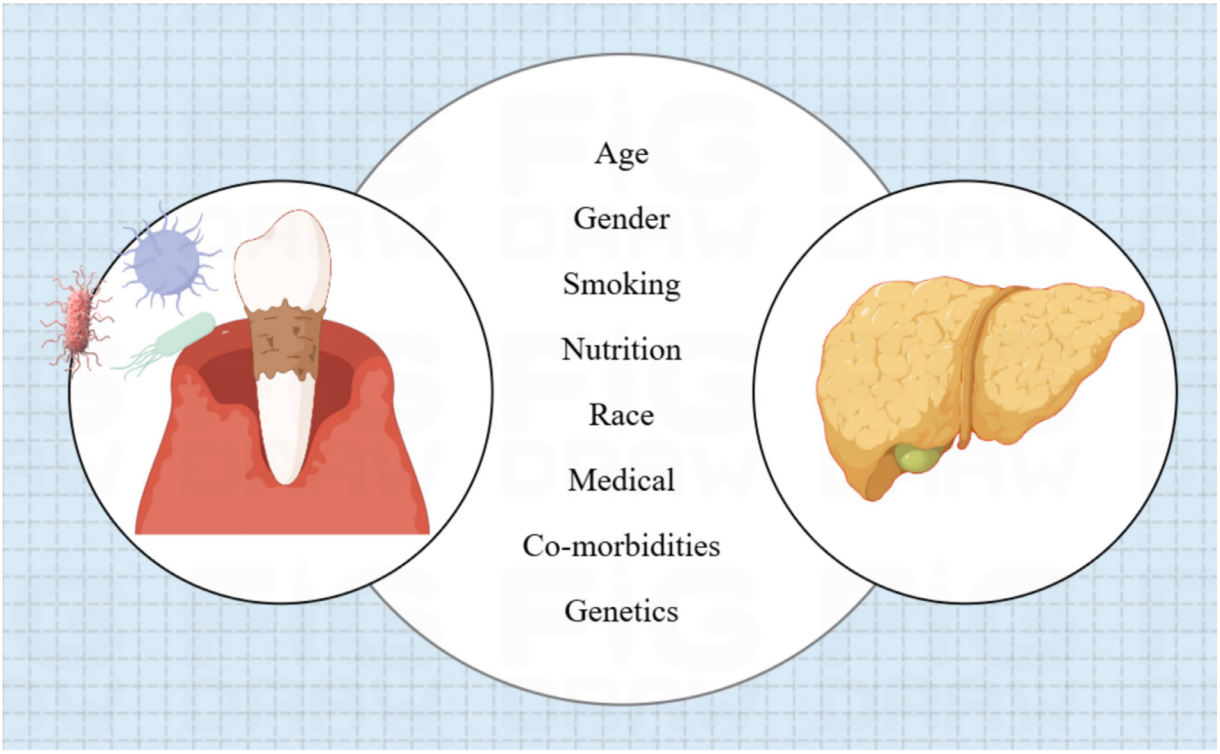

Fig. 2 – Common risk factors between NAFLD and periodontitis. This schematic presentation is based on data reported in a systematic review with a meta-analysis.[10]

correlation may be caused by many factors, among which the mechanism of periodontal pathogens may be associated with the secretion of OMVs or systemic inflammation and oxidative stress,[47,48] which needs further study.

Periodontal Pathogens and Their OMVs

Periodontal pathogens

Periodontal pathogens are a subset of the oral microbiome closely associated with various oral diseases. Key periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* contribute to periodontal diseases by secreting toxins and disrupting immune responses.[49] These bacteria proliferate in subgingival biofilms, leading to gingivitis and the formation of periodontal pockets and eventually causing tooth mobility or even loss.[50] *P. gingivalis* forms the 'red complex' with *Tannerella forsythia*, and *Treponema denticola*, which plays an important role in periodontal pathogens and promotes the occurrence and development of chronic periodontitis.[51]

Besides, bacteria residing in the periodontal pocket possess a pathway to systemic dissemination via the inflamed epithelium, entering the bloodstream and travelling to distant body sites.[52] *P. gingivalis* has been identified in extraneous sites, including synovial fluid and plasma, which underscores its potential link with systemic conditions.[18] This bacterium has also been shown to interact with host systems, promoting pathways associated with Alzheimer's disease, diabetes, cardiovascular disease, and may even exacerbate central nervous system inflammation, predisposing individuals to various diseases.[19] In a case report, a 54-year-old morbidly obese woman with NASH and chronic periodontitis died of sepsis caused by infection with *P. gingivalis*, and *P. gingivalis* was remarkably found in her hepatocytes,[53] representing the significance of *P. gingivalis* in the progress of NAFLD.

In recent years, accumulating evidence has increasingly highlighted the significant roles that oral pathogenic bacterial OMVs play in the pathogenesis of oral and systemic disease.[54]

Outer Membrane Vesicles

OMVs are spherical nanoparticles ranging from 10 to 300 nm in diameter constitutively produced by Gram-negative bacteria.[55,56] The biogenesis mechanism is highly conserved.[57] It involves multiple steps, including an accumulation of phospholipids in the outer membrane, asymmetric expansion of outer membrane, budding and release. Studies have shown that the production of OMV is closely related to the growth state of bacteria, environmental factors and specific gene expression.[55,56] The proportion of cells to OMVs has been estimated to be approximately 1:2000.[57]

As a sophisticated secretion system, OMVs ferry a diverse cohort of molecular agents including nucleic acids, proteins, lipids, virulence factors, toxins and metabolites.[58] These vesicles are significant not only in terms of bacterial pathogenicity but also in terms of survival and virulence. Notably, existing research has shown an association between active

virulence factors and toxins with OMVs, indicating OMVs play a significant role in the virulence of Gram-negative pathogens.[59] Due to encapsulation within OMVs, virulence factors are shielded from proteolytic degradation. Thus, their delivery is enhanced over distances, and their release can be synchronised with other bacterial effectors.[27]

OMVs play a role in the formation and maintenance of bacterial biofilms in the oral cavity, providing a protective layer that enables bacteria to resist antibiotic treatment and host immune attacks.[58] Furthermore, OMVs can induce pathological reactions in organs distant from the original site of infection, thereby promoting the development of systemic diseases.[60] For example, bacteria-derived OMVs may trigger atherosclerosis by promoting inflammation and damage to arterial endothelial cells, leading to a range of cardiovascular diseases.[61] In addition, a significant factor contributing to Alzheimer's disease is that OMVs can cross the blood-brain barrier, allowing the carried toxins to exacerbate brain inflammation and neurodegenerative changes.[62]

Periodontal pathogens–derived OMVs

OMVs regulate the host's immune response by either activating or suppressing immune cells, which is particularly crucial in periodontal disease.[58] As mentioned before, periodontitis is closely related to a group of red complex bacteria, *P. gingivalis, T. forsythia* and *T. denticola*. To date, *P. gingivalis* OMVs are the most studied. OMVs derived from *P. gingivalis* are beneficial to the interaction of other oral pathogens and can regulate the virulence of oral microflora. *P. gingivalis* OMVs can not only promote the horizontal gene transfer between *P. gingivalis* strains to prevent biofilm formation[63] , but also participate in the co-aggregation of other oral pathogens, including *Treponema Denticola* and *Lachonanaerobaculum saburreu*.[64] More importantly, *P. gingivalis* OMVs are internalised by the host epithelial cells through a Rac1/lipid raft-dependent mechanism, independent of dynamin, caveolin and clathrin pathways, so that the early endosomes containing OMVs can be co-located with lysosomal markers within 90 minutes, thus avoiding the risk of degradation and increasing the presence of acidified compartments.[65] However, in nonphagocytic cells, *P. gingivalis* OMVs internalise through lipid rafts and recognise the host cytoplasmic receptor NOD1 of bacterial peptidoglycan to activate the proinflammatory reaction, which stimulates NF-κB proinflammatory transcription factors and produces proinflammatory cytokines such as IL-8.[66] Also, *P. gingivalis* OMVs can be used as a carrier of virulence factors that destroy normal cell functions by effectively invading various types of host cells.[67]

Recent findings identified gingipain-positive cells within the liver of mice following intraperitoneal administration of *P. gingivalis* OMVs, and the hepatic glycogen synthesis induced by insulin was reduced, suggesting *P. gingivalis* OMVs could attenuate insulin sensitivity by delivering gingipains to the liver.[68] Further mechanism study found that the gingipains in *P. gingivalis* OMVs impeded insulin signalling through the Akt/GSK-3β pathway in a gingipain-dependent manner.[68]

Additionally, *Fusobacterium nucleatum* has emerged as a critical driver of periodontitis. *F. nucleatum* OMVs have been shown to induce oxidative stress and promote the production of proinflammatory cytokines. *In vivo* and *in vitro* studies identified that *F. nucleatum* OMVs could damage the periodontal tissue in mice by leading to apoptosis of mouse gingival fibroblasts and switching macrophages from M0 to M1 phenotype.[69] Engevik et al. found that outer membrane vesicles from *F. nucleatum* can exacerbate intestinal inflammation and stimulate IL-8 and TNF expression in colonic epithelial cells.[70] Remarkably, in certain cases, *F. nucleatum* originating from the oral cavity has been associated with severe liver abscesses, potentially entering the liver via bacteremia or the gastrointestinal route, and ultimately contributing to cirrhosis.[71]

Potential Link Between Periodontal Pathogens –Derived OMVs and NAFLD

Periodontal pathogens–derived OMVs and cells in the liver

Liver, the largest solid organ in the body, plays a principal role in clearing bacteria and pathogens derived from the bloodstream. Kupffer cells, macrophages, hepatic stellate cells and hepatocytes are essential in this clearance mechanism (Figure 3).[72]

Kupffer cells, the liver-resident macrophages, are essential for maintaining liver homeostasis and responding to infections.[73] Periodontal pathogen–derived OMVs can activate Kupffer cells, leading to the secretion of proinflammatory cytokines and chemokines such as IL-6, TNF-α, and MCP-1.[74] This activation results in an inflammatory cascade that exacerbates liver inflammation and promotes the progression of liver diseases such as NASH and cirrhosis.[75] Chronic activation of Kupffer cells by OMVs can induce a sustained inflammatory response, contributing to liver fibrosis and impairing liver function.[76] Also, OMVs produced by periodontal pathogens have profound effects on monocytes and macrophages. These OMVs can be internalised by monocytes and macrophages, leading to the activation of various signalling pathways.[77] This activation stimulates not only the production of proinflammatory cytokines, including TNF-α, IL-1β and IL-6, but also the oxidative stress reaction of cells which play a critical role in the inflammatory response.[78] The increased production of these cytokines can enhance systemic inflammation, contributing to the pathogenesis of chronic inflammatory conditions, including liver diseases.[79] Additionally, OMVs from periodontal pathogens can influence macrophage polarisation, promoting a shift towards a proinflammatory M1 phenotype, which further exacerbates tissue inflammation and damage.[80]

Hepatic stellate cells (HSCs) are central to the development of liver fibrosis. In their quiescent state, HSCs store vitamin A, but upon activation they transform into myofibroblast-like cells that produce excessive extracellular matrix components, leading to fibrosis.[81] Periodontal pathogen–derived OMVs can upregulate the expression of *smad2/3* gene by activating TGF-β pathway, thus inducing the activation of HSCs, inducing their transformation into a fibrogenic state.[82] This activation can also occur indirectly through the inflammatory environment created by activated Kupffer cells and other immune cells responding to OMVs.[83] The fibrogenic

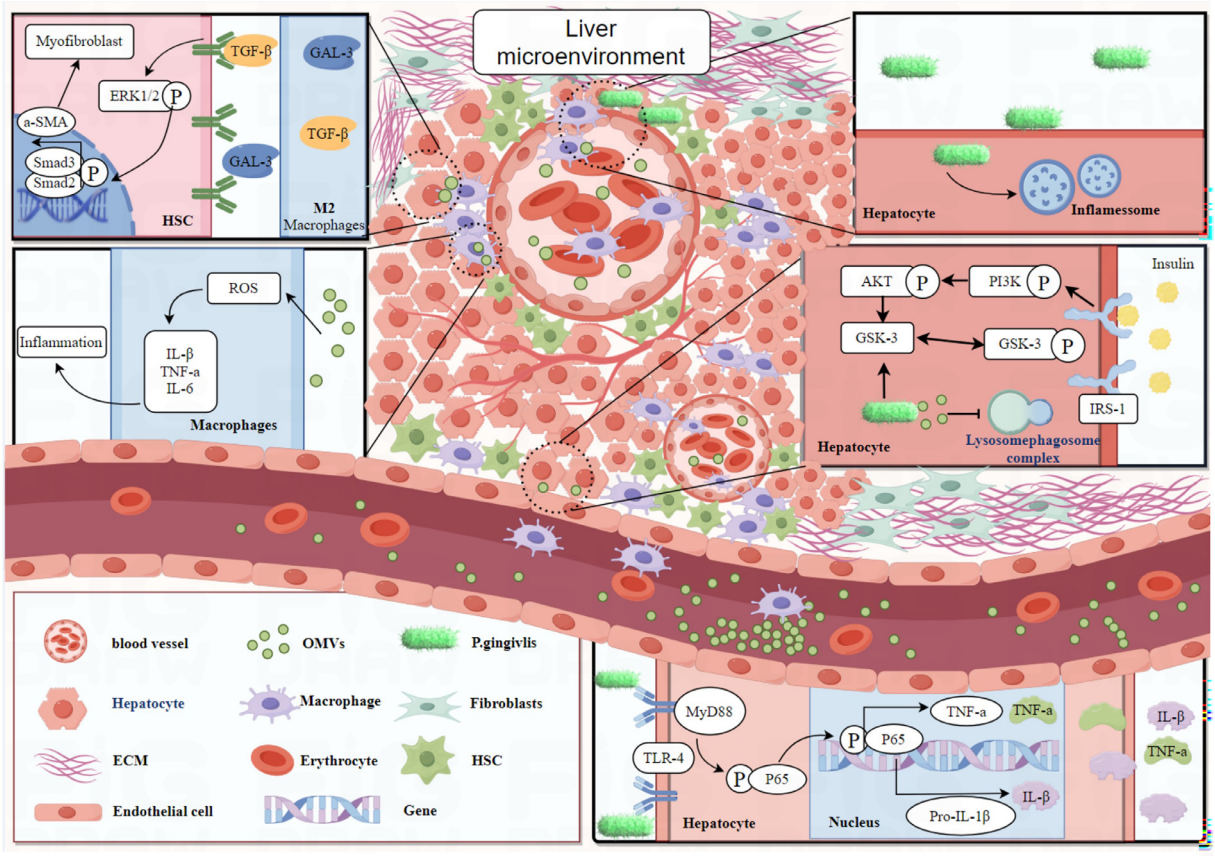

Fig. 3 – Mechanisms of OMVs influence on hepatic pathology. This figure illustrates the dynamic interactions between OMVs, macrophages, hepatic stellate cells and hepatocytes within the liver environment. It highlights the process of OMV-mediated modulation of cellular pathways leading to hepatic fibrosis, including the activation of hepatic stellate cells, enhancement of pro-inflammatory cytokine production and stimulation of fibrogenesis. This visualisation underscores the multifaceted role of OMVs in the progression of liver diseases, from initial cellular interaction to the eventual pathological outcome.

activity of HSCs driven by OMVs contributes significantly to the development and progression of liver fibrosis and cirrhosis.[84]

Hepatocytes, the primary functional cells of the liver, are responsible for crucial metabolic, detoxification, and synthetic functions.[85] Periodontal pathogen–derived OMVs can interact directly with hepatocytes, causing cellular stress and apoptosis.[86] In this process, the PI3K-AKT signalling pathway and its downstream GSK-3β target gene play an important role in lipid accumulation in NAFLD and promoting liver fibrosis. Periodontal pathogen OMVs not only promote liver fibrosis by activating the gene expression of GSK-3β target but also inhibit the formation of lysosomes to prevent their own functional failure.[87] Moreover, the low expression of TLR4 and signal transduction molecules may help to enhance the tolerance of the liver to continuous exposure to intestinal microflora.[88] On the contrary, the expression of TLR4 and its signal transduction molecules in hepatocytes is upregulated by periodontal pathogen OMVs, leading to inflammatory reaction, oxidative stress and fibrosis of hepatocytes. Furthermore, the inflammatory environment created by the interaction of OMVs with immune cells exacerbates hepatocyte damage, promoting conditions such as NAFLD.[89]

The relationship between oral pathogenic bacterial OMVs and NAFLD

The relationship between oral pathogenic bacterial OMVs and NAFLD has been confirmed to be close.[90] Bacterial endotoxins and other inflammatory molecules carried by oral bacterial OMVs can activate liver macrophages, triggering hepatic inflammatory responses and promoting the progression of liver fibrosis.[91] Enzymes such as proteases and endotoxins can directly damage liver cells, leading to cell death or functional impairment.[92] More importantly, oral pathogenic bacteria–derived OMVs can alter the hepatic immune micro-environment by affecting the activity of T cells and other immune cells, influencing the progression of liver diseases.[93]

Given that *P. gingivalis* OMVs contain a substantial amount of lipopolysaccharides (LPS) within their outer membrane, it follows that *P. gingivalis* OMVs are predisposed to integration and accumulation, especially within hepatic tissue. Recent evidence indicates that mice receiving *P. gingivalis* OMVs for 4 weeks exhibit gingipain-positive cells within their hepatic sinusoids, which implies that *P. gingivalis* OMVs are primarily phagocytosed by liver macrophages via LPS. Seyama et al.

observed gingipain-positive cells in the livers of mice administered with *P. gingivalis* OMVs, confirming hepatic exposure to gingipains encapsulated within *P. gingivalis* OMVs.[68] Nevertheless, the literature on the interplay between periodontal pathogen OMVs and NAFLD remains scant.

Recent studies have proved that *P. gingivalis* OMVs can invade gingival keratinocytes in a dose-dependent manner, causing oxidative stress.[94] In addition, they may lead to the long-distance transportation of periodontal pathogens OMVs through bloodstream route or oral-gut-liver axis, and aggravate systemic diseases such as cardiovascular disease or diabetes by generating oxidative stress.[23] Therefore, whether the periodontal pathogen OMVs affect the occurrence and development of NAFLD through oxidative stress mechanism needs further research to confirm.

Possible routes of oral pathogen–derived OMVs to the liver

One pathway for oral pathogenic bacterial OMVs to enter the liver is the bloodstream route, where bacteria-derived OMVs can directly enter the microvasculature through damaged oral mucosa and thus reach various organs via the blood circulatory system (Figure 4).[95] In the presence of oral inflammation such as periodontitis, inflammatory responses may facilitate the penetration of bacteria-derived OMVs through the vascular walls into the bloodstream.[96] Furthermore, oral pathogenic bacterial OMVs may interact with white blood cells (such as macrophages and neutrophils) migrating to the site of inflammation and could be transported to other parts of the body along with these cells.[96] However, the dynamic distribution of these vesicles in each organ is still unknown. More research is needed to investigate how OMVs affect systemic inflammation and contribute to the pathogenesis of liver diseases.

The other pathway is the oral-gut-liver axis that has been proposed recently (Figure 5). According to this theory, oral pathogenic bacterial OMVs enter the gut through oral swallowing and affect systemic diseases by altering the microenvironment of the gut microbiota.[97] When the OMVs of oral pathogenic bacteria enter the stomach, the micro-environment of the gastric flora is thrown out of balance. Consequently, the secretion of gastric acid is reduced, and the gastric mucosal barrier is destroyed. This provides convenient conditions for oral pathogenic bacteria to enter the intestinal circulation subsequently.[8] Previous studies have shown that the ecological imbalance caused by intestinal translocation of oral bacteria may be related to the pathogenesis of NAFLD. Blasco-Baque et al. found that mice fed a high-fat diet and orally inoculated with *P. gingivalis, Clostridium nucleatum,* and *Prevotella intermedium* showed insulin resistance and impaired blood glucose metabolism.[98] However, there was no significant change in the intestinal microflora of the treated mice. Ohtsu et al.[99] reported that *P. gingivalis* and its OMVs significantly increased the expression of

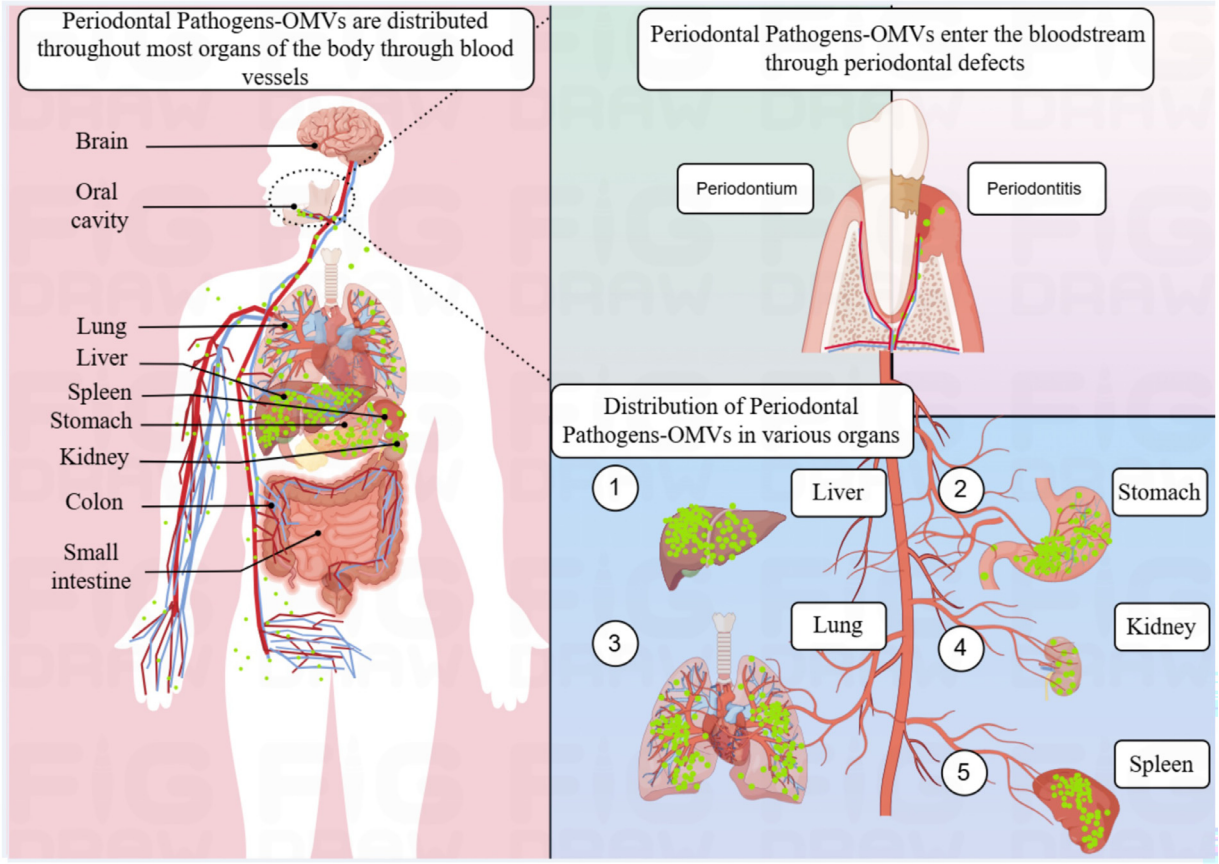

Fig. 4 – Diagram of the systemic blood route distribution of OMVs. The figure describes the circulatory pathway of adventitial OMVs derived from oral pathogens to various organs. The key processes include the transfer of microbial components from the oral cavity to blood vessels and their subsequent effects on the pathogenesis of organ injury.

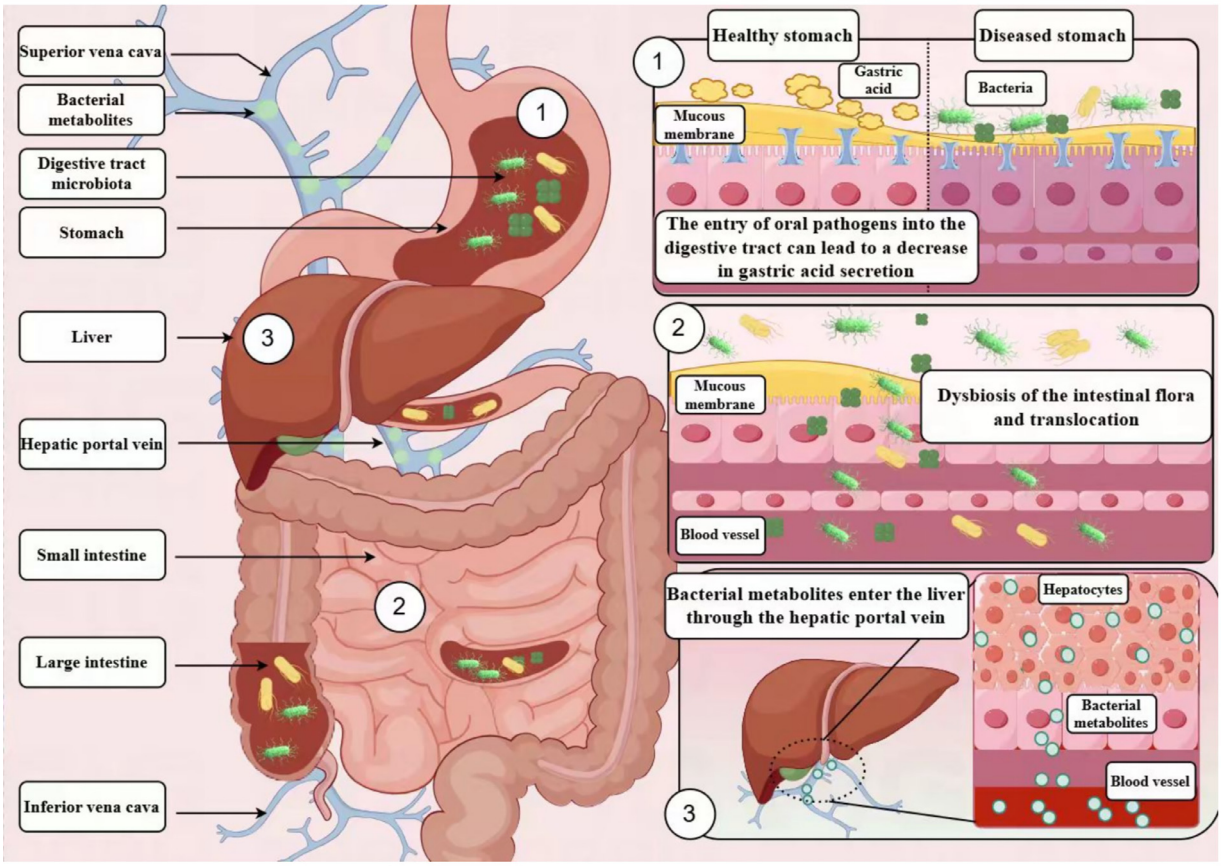

Fig. 5 – Illustration of the oral-gut-liver axis. This figure depicts the complex interactions between oral microbiota, the gut microbiome and liver health and the path of oral pathogen–derived OMVs. Key processes include the translocation of oral microbial components to the gut and their subsequent influence on the gut-liver axis.

inflammatory factors, such as TNF-α and C-C motif chemokine ligand 2, in diabetic mice induced by streptozotocin. However, it has little effect on intestinal microflora without inhibiting tight junction protein. On the contrary, several animal studies have shown that oral administration of periodontal bacteria, including *P. gingivalis* and *Actinobacillus actinomycetemcomitans*, is related to changes in intestinal microflora and glucose and lipid metabolism pathways, which in turn lead to insulin resistance and liver fat deposition.[100,101] These studies indicated that the intestinal ecological imbalance induced by *P. gingivalis* and its products further downregulated the gene expression of tight junction proteins involved in intestinal barrier function and increased the serum lipopolysaccharide level.[100,102] Therefore, further animal studies are needed to prove the long-distance transmission of *P. gingivalis* OMVs.

The Effect of Periodontal Treatment on the NAFLD

Owing to the strong relation between periodontal pathogens and NAFLD, patients with NAFLD should pay more attention to maintaining their oral hygiene. Recently, toothbrushing, the most common method of reducing oral pathogens, has been investigated in relation to NAFLD.[103] In this study, 6,453 adults were divided into three groups according to toothbrushing frequency (≥3/day, ≥2/day and ≤1/day), and the prevalence of NAFLD was investigated. The results indicated that higher frequency of toothbrushing was inversely related to NAFLD, suggesting regular toothbrushing may decrease the risk of NAFLD. There are few clinical studies to investigate the effect of periodontal therapy on NAFLD results. Yoneda et al.[104] conducted an intervention study to evaluate whether periodontal therapy can regulate the biochemical parameters of NAFLD patients with periodontitis, and reported the decrease of ALT and AST levels within 3 months after periodontal therapy. Recently, Kamata and his colleagues published the results of a multicentre randomised clinical trial to evaluate whether periodontal therapy can reduce liver injury and endotoxemia in patients with NAFLD, which showed that periodontal treatment (scaling and root planing) could induce significant short-term (12 weeks) and mid-term (60 weeks) reductions in liver enzyme levels and *P. gingivalis* antibody titres.[105,106]

The newly established oral-gut-liver axis reveals that the prevention and treatment of NAFLD is achievable by reducing oral pathogens and correcting intestinal microbial imbalance. One approach is the use of antibiotics, prebiotics and probiotics to improve the balance of intestinal microbial flora.[105,107] A recent study demonstrated that curcumin (a turmeric root extract) significantly suppressed the gene expressions of IL-6,

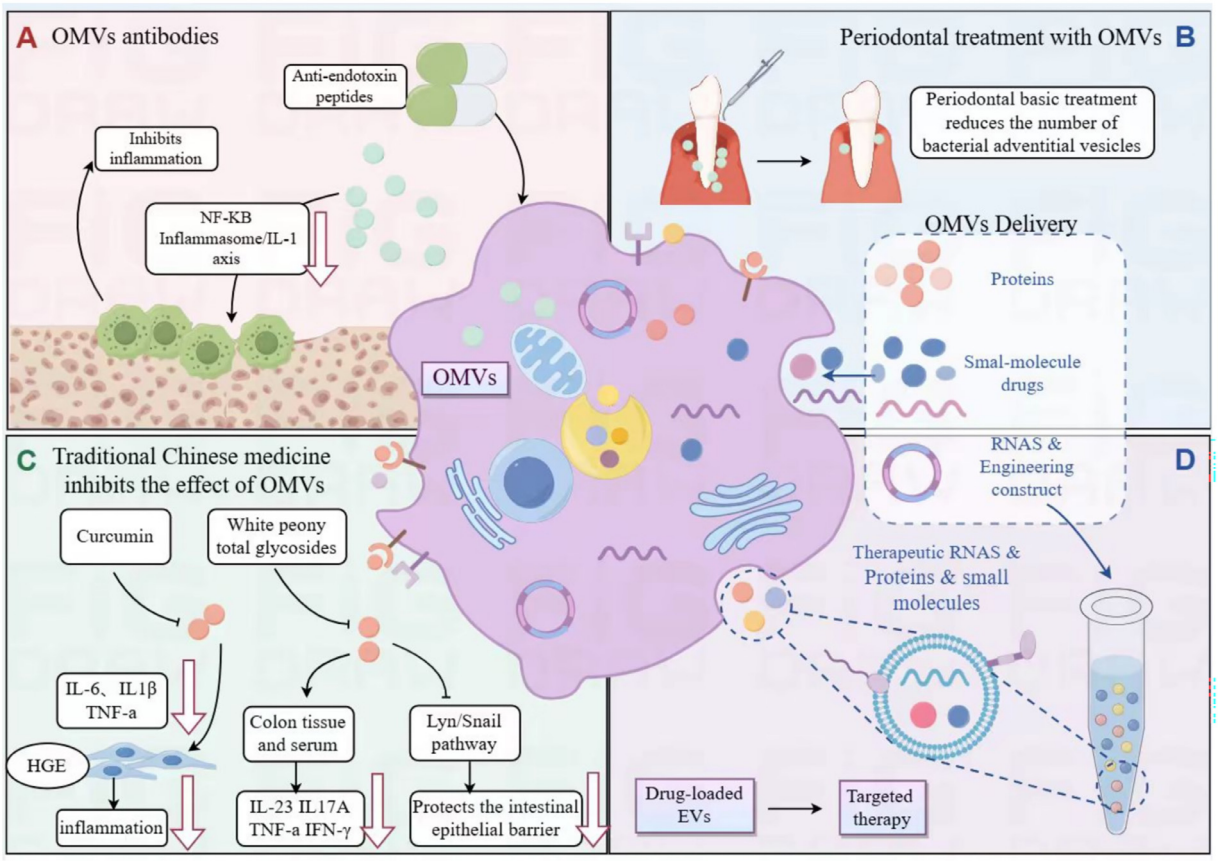

Fig. 6 – Innovative treatment and future prospect of periodontitis complicated with NAFLD. The innovative treatment plan encompasses four main aspects: (a) using antibiotics and probiotics to inhibit inflammation in nonalcoholic fatty liver disease; (b) reducing bacterial outer membrane vesicles through periodontal treatment; (c) using traditional Chinese medicines (e.g. curcumin and paeony glucosides) to inhibit inflammatory pathways; and (d) delivering therapeutic RNA, proteins and small molecules via OMVs for targeted therapy.

IL-1β and TNF-α in human gingival epithelial cells stimulated by *P. gingivalis* OMVs. Additionally, curcumin mitigated the cytotoxic effects, intracellular invasion and cellular apoptotic death effects of OMVs in a dose-responsive manner.[108] These findings highlight the role Chinese medicine components can play in the normal biological function of the OMVs of oral pathogenic bacteria, suggesting their potential efficacy in preventing periodontal disease and NAFLD.

Since *P. gingivalis* plays an important role in the interaction between oral bacteria and NAFLD, targeting it may serve as a new strategy for the treatment of NAFLD, such as using anti-CR3 receptor drugs to reduce bacterial adhesion,[109] using gingival protein inhibitors to alleviate periodontitis and related systemic diseases,[110] using AMPs to prevent *P. gingivalis* from adhering to host cells[111] and regulating the production of OMVs to prevent biofilm formation.[112] Pfalzgraff et al. have demonstrated that the synthetic anti-endotoxin peptide Pep19-2.5 effectively reduced the *Escherichia coli* OMVs –induced inflammatory responses.[113] However, the research on these drugs in NAFLD related to pathogenic oral bacteria such as *P. gingivalis* is preliminary and requires further study (Figure 6).

Conclusions

NAFLD is an emerging global health threat, and a series of epidemiological studies have shown that there is a potential link between NAFLD and periodontal disease. Among them, periodontal pathogens OMVs have gradually become an important promoting factor.[114] This review indicates that periodontal pathogens OMVs can be transported to the liver, but the specific pathway is still controversial. This underscores the need for continued research to develop new, optimised schemes to determine the forms of systemic distribution.

Moreover, strengthening oral hygiene and strategic management of pathogenic oral bacteria represent effective interventions for NAFLD related to bacterial infection. Our review summarises the related treatment methods and suggests that continued research efforts should focus on how to control the number and scope of periodontal pathogens OMVs to provide professionals with new clinical combined treatment guidelines and potential therapeutic targets for periodontitis complicated with NAFLD. On the other hand, these research efforts could improve the

therapeutic effectiveness for patients with periodontitis complicated with NAFLD.

Finally, the review shows that periodontal pathogenic OMVs may promote the occurrence and development of cardiovascular diseases and diabetes by generating oxidative stress in the body, so it is worth further study and discussion to determine the relevant mechanism to promote the occurrence and development of NAFLD.

Author contributions

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Conflict of interest

The authors declare no conflict of interests.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2025.03.029.

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