Author's Point-by-Point Responses to the Reviewers' Comments

I have rewritten the manuscript as described below according to the reviewers' comments. All revised points in the manuscript are highlighted in yellow.

Q; Question A; Answer

Reviewer #1

Method:

Q-1

No details were provided on the methodology used to write this review. It is necessary to describe the methodology justifying the exhaustiveness of the work. Was the PRISMA methodology used? Is there a Flowchart?

A-1

We chose the narrative review format for this paper to provide a comprehensive summary of the large body of evidence on periodontal disease and NAFLD provided by a wide range of in vivo, in vitro, epidemiological, and clinical studies, with as yet no consistent consensus and variations. Thus, there is no flowchart and PRISMA for systematic article search.

2. Periodontal disease as a risk factor for systemic diseases

2-1. Diabetes

Q-2

It would be interesting to know the prevalence of periodontitis in diabetic patients and vice versa; is the prevalence higher compared to the general population? Are there data in the literature?

A-2

Yes, there is data in the literature on this topic and this has been added to the text, "Furthermore, meta-analyses also indicate a higher prevalence and severity of periodontal disease in diabetic patients and vice versa [87, 88]"

Q-3

P5: “modulating bacterial-mucosal immunity-inflammation may alleviate type 2 diabetes”

➔ Please, specify by what mechanisms and the impact on periodontitis

A-3

This has now been described and referenced in the text (Li et al., 2018) as follow.

“Furthermore, intervening with the flora or modulating bacterial-mucosal immunity-inflammation may alleviate type 2 diabetes; the later by mechanisms that include modulation of T lymphocytes [102],[103].”

Q-4

P6: “In addition, although the diversity of the subgingival and supragingival microbiome decreases when subjects with type 2 diabetes are compared to normoglycemic individuals, the bacterial shift in individuals with periodontitis is less prominent in type 2 diabetes subjects than in normoglycemic individuals”

➔ One hypothesis mentioned in the review, justifying these results, is the modification of the oral microbiota by tobacco. What other factors are known to alter the oral microbiota? tooth brushing?

- Immune modifications and the associated inflammation are an integral part of the association of these 2 pathologies. Thus it would be interesting to present the inflammatory status associated with diabetes but also with the metabolic syndrome and to discuss similarities with the inflammatory status of periodontitis.
- Furthermore, it would also be interesting to briefly discuss the specificity of periodontitis in the onset of metabolic diseases. Indeed, can other inflammatory diseases, infections also be associated with the onset of metabolic diseases?
- It would also be interesting to describe the modifications in the gut microbiota observed during diabetes but also metabolic syndrome or obesity.

A-4

Data/associations could not be found relative to the role of toothbrushing effects on the oral microbiota in the context of diabetes and periodontal disease (Almedia-Santos et al., Front Microbiol. 2021 Feb 5;12:610370).

"Studies suggest a relationship between the inflammatory status associated with diabetes or metabolic syndrome and the inflammatory status of periodontal disease [93, 94]. Indeed chronic tissue inflammation has been recognized in the onset of metabolic diseases [95]." This has been added to the text.

The modifications in the gut microbiota observed during diabetes or metabolic syndrome have already been discussed and referenced in the text; the reader is directed to these reviews for a detailed exploration of these topics:

"Much attention has been given to the ability of chronic conditions to alter the microbiome. For instance, individuals with type 2 diabetes or obesity have a modified gut microbiome (reviewed by [97-100]). Conversely, mice treated with *Enterobacter cloacae* B29, which was isolated from the intestines of obese patients with diabetes, also develop obesity and insulin resistance [101]; showing that microbes can also directly induce diabetes-related symptoms."

"I In recent years, much interest has been given to the microbiome. Metabolic diseases alter the gut microbiome (reviewed by [100]), and it is well known that the oral microbiome varies significantly between healthy and periodontitis patients [169]. Additionally, alterations in the gut microbiome have been linked to obesity and metabolic syndrome [170]. Furthermore, obesity can alter the oral microbiome of individuals with type 2 diabetes [112] and it can reduce microbial diversity in the distal gut [171, 172]."

2-2. Metabolic syndrome

Q-5

P8:

"Metabolic diseases alter the gut microbiome"

➔ Could modifications of the gut microbiota also be at the origin of metabolic diseases?

"obesity can alter the oral microbiome of individuals with type 2 diabetes"

➔ idem, could modifications of the oral microbiome also be at the origin of the onset of obesity?

“Additionally, alterations in the gut microbiome have been linked to obesity and metabolic syndrome”

➔ Please specify what these alterations are?

A-5

The text already references reviews and literature on the topic of the gut microbiome and metabolic syndrome. The readers are directed to these reviews for a detailed exploration of these topics.

An additional information has been added to the text in the new manuscript as follow.

“In addition, there are a growing number of studies suggesting potential links between the gut or oral microbiome and obesity [174-176]”.

3. Relationship between periodontal disease and NAFLD

Q-6

P9:

“The relationship between periodontitis and NAFLD is supported by a number of epidemiological studies”

➔ Please add references.

“Cross-sectional studies have … for estimating the degree of fatty liver and liver fibrosis”

➔ Please add references.

“Akinkugbe et al. followed 2,623 non-NAFLD … was diagnosed by abdominal ultrasonography and serum ALT levels”.

➔ Please add reference. Can you complete with statistical indicators?

“In a 13-year cohort study of 6,165 subjects participating in a Finnish population-based health study, Helenius-Hietala et al. found a positive correlation between the proportion of sites with deep periodontal pockets and the hazard ratio for the development of severe liver disease.

➔ Please add reference

A-6

In accordance with your instructions, references have been added to the new manuscript. The following text regarding the Akinkugbe et al. paper has been inserted on page 9.

“Compared to subjects without CAL ≥3 mm, the incidence of NAFLD was significantly higher in patients with <30% or ≥30% affected sites with CAL ≥3 mm. The adjusted

incidence rate ratio for NAFLD was 1.28 for <30% and 1.60 for ≥30% of affected sites with a statistically significant difference, respectively. Similarly, the incidence rate difference was statistically significant at 5.49 for < 30% of sites and 11.11 for ≧30% of sites."

4. Mechanisms by which periodontal disease exacerbates NAFLD based on the Oral-Gut-Liver axis

Q-7

P11: " Kawamoto et al. [16] found that fecal samples from patients with severe periodontitis were enriched in Acidaminococcus, Clostridium, Lactobacillus, Bifidobacterium, Megasphaera, and Romboutsia compared to those from healthy subject"

➔ It would be particularly interesting to comment on the modifications of the fecal microbiota observed during periodontitis with those during NAFLD, obesity, diabetes...

A-7

Currently, studies investigating the relationship between periodontal disease and gut microbiota in humans are in their early stages and do not provide sufficient evidence for consistent results. Therefore, we have mentioned it in section 4 in new manuscript as follows.

"However, few studies have observed changes in the gut microbiota in patients with periodontal disease, and consistent trends in the gut dysbiosis remain unclear, with wide variations. In the future, it would be interesting to compare the modifications of the gut microbiota in the state of periodontal disease with those observed in NAFLD, diabetes, and obesity to elucidate the complex relationship between periodontal disease and systemic diseases."

5. Periodontal disease from the viewpoint of oral dysbiosis

Q-8

P13: What about the red and orange complex observed in periodontitis in metabolic pathologies (diabetes, metabolic syndrome)?

A-8

In accordance with your suggestion, the following sentences regarding the relationship between red and orange complex and periodontitis in metabolic pathologies has been

inserted on page 13.

"Periodontitis has been reported to be associated with many systemic diseases [241], especially diabetes caused by metabolic syndrome. The prevalence of periodontitis in diabetic subjects was higher than that in non-diabetic subject [242]. In addition, poorly controlled diabetic subjects have a more severe periodontitis [243]. In periodontitis sites, a higher quantity of *P. gingivalis* in poor controlled diabetic subjects was observed than that of non-diabetic subject [244]."

Q-9

P14: T forsythia ➔ *T. forsythia*

A-9

Section 5, Page 14: "T forsythia" was replaced for "*T. forsythia*".

Q-10

It would be interesting to develop this part by describing in particular the similarities observed in the dysbiosis of the oral and gut microbiota. It would thus perhaps be more judicious to integrate this part after the description of the oral / gut dysbiosis during periodontitis and NALFD

A-10

Thank you for your suggestion. As we answered in Q7, unfortunately, we don't have a view showing consistent changes in the gut microbiota in patients with periodontal disease between studies; we have added a comment on this point on Page 9.

Q-11

What about *P. gingivalis* in NAFLD patient without periodontitis?

A-11

To our knowledge, no studies have investigated whether the presence or absence of periodontal disease affects the proliferative activity of P. gingivalis in patients with NAFLD. However, it is generally known that the frequency and relative abundance of *P. gingivalis* is increased in patients with periodontal disease compared to those without periodontal disease, and a similar trend may be observed in patients with NAFLD. Furthermore, Kamata et a.(2022) reported that periodontal therapy reduces serum anti-*P. gingivalis* antibodies in NAFLD patients, providing limited support for this hypothesis.

7. Relationship between gut dysbiosis and NAFLD

Q-11

* Are there data on oral dysbiosis of NAFLD patients?: specify

A-11

There is limited data examining oral dysbiosis of NAFLD patients. One of the largest study included 150 NAFLD patients and compared them to 60 non-NAFLD control patients. They saw that patients with biopsy proven biopsy were more likely to have higher levels of *P. gingivalis* in the oral microbiome as compared to non-NAFLD controls (Yoneda et al. 2012. PMID 22340817). This has now been described and referenced in the text (Section 3, P10) your as follow.

"Yoneda et al [37] analyzed various periodontopathic bacteria in saliva collected from non-NAFLD control subjects and NAFLD patients using PCR assays and showed that the detection frequency of P. gingivalis was significantly higher in the NAFLD patients."

8. Periodontal approaches in the prevention and treatment of NAFLD

8-1. Management of oral microbiota with conventional periodontal treatment

Q-12

P18:

"Yoneda et al 37 reported that in a single-arm intervention study,…..led to a significant improvement in AST and ALT at 3 months after treatment."

➔ Did this study also investigate markers of glucose metabolism?

A-12

Yes. In this study, they evaluated glucose metabolic parameters, including serum levels of glucose and insulin and insulin resistance, and found no significant differences between the two groups at 12 and 60 weeks after periodontal treatment. We have mentioned it in section 8 (Page 19) in new manuscript as follows.

"The SRP group showed significantly greater reductions in blood lipid parameters, including total cholesterol, LDL-cholesterol, and triglycerides at 12 weeks post-treatment, while no significant changes in glycometabolism parameters such as

glucose and insulin."

Q-13

P19:

"prebiotics are defined as fermented foods containing dietary fiber that activate intestinal bacteria and probiotics"

➔ Activation but also modifications in the composition of intestinal bacteria. What are the consequences of the proposed treatments on systemic inflammation?

A-13

As discussed on Page 19, the proposed microbiome-targeted therapy could attenuate inflammatory conditions and improve organ function in diseases such as inflammatory bowel disease and NASH. In accordance with the suggestion, the text in section 8 (Page 19) of the new manuscript has been revised as follows.

"prebiotics are defined as fermented foods containing dietary fiber that activate intestinal bacteria and probiotics and modify composition of microbiome".

9. Conclusion

Q-14

P21:

"… induces gut dysbiosis and is involved in the pathogenesis of NAFLD"

➔ also oral dysbiosis

A-14

We have revised the section 9 (Page 21) in new manuscript as follows.

"A growing body evidence from multiple angles proposes that periodontal disease, accompanied by oral inflammation and pathological changes in the oral microbiome, induces gut dysbiosis and is involved in the pathogenesis of NAFLD"

Reviewer #2

The title is well written, precise, and meet the objectives of the study. There are some shortcomings in the abstract, last lines in the abstract need to improve with the special concern how this review is beneficial for human beings.

Abstract

Q-1

Mention the name of compounds.

A-1

"..other volatile organic compounds such as acetone, phenol and cyclopentane"

Q-2

Add conclusion of the review and future recommendations here.

A-2

The text corresponding to the conclusion of the review and future recommendations is mentioned in abstract (Page 2) of the new manuscript as follows.

"In conclusion, it is presumed that the pathogenesis of NAFLD involves a complex crosstalk between periodontal disease, gut microbiota, and metabolic syndrome. Thus, the conventional periodontal treatment and novel microbiome-targeted therapies that include probiotics, prebiotics and bacteriocin would hold great promise for preventing the onset and progression of NAFLD and subsequent complications in patients with periodontal disease."

8-1. Management of oral microbiota with conventional periodontal treatment

Q-3

Why author correlate oral microbiota with conventional treatment?

A-3

The reasons for the involvement of conventional periodontal treatment in the oral microbiota are mentioned in Section 8-1 (Page 18) of the new manuscript as follows.

"Periodontal treatment is based on the mechanical and chemical removal of dental plaque and pathogenic factors from the root surfaces by the patient or by a specialist to eliminate inflammation in the periodontal tissues and promote wound healing in the host. It is known that patients who achieve good oral hygiene and a healthy morphology of periodontal tissues through periodontal treatment show a decrease in the total number of bacteria and the proportion of periodontal pathogens in saliva and plaque, as well as a change in the composition of the oral microbiota to a healthy state."

9. Conclusion

Q-4

Conclusion should be concise.

A-4

According to the author guidelines for review article, in the Conclusion, the WJG requires that we summarize the full text, give future perspectives, put forward reasoned opinions and suggestions, and put forward the author's own views, indicating what the author is for and against. Thus, we have included in this section a summary of this review, and critical discussion, as well as mention of future work.