

Dear editor and reviewers,

Thank you very much for your letter and the comments from the referees about our paper (Manuscript NO.: 73159) submitted to "*World Journal of Gastroenterology*". We very much appreciate the careful reading of our manuscript and valuable suggestions of the reviewer. We have carefully checked the manuscript and revised it according to the comments. We also responded point by point to each reviewer comments as listed below.

If you have any question about this paper, please don't hesitate to let me know.

Sincerely yours,

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The following is a point-to-point response to the reviewers' and editor's comments.

**Reviewer #1:**

It was a pleasure going through the manuscript. It is well designed and well scripted. However, there are few issues which I am commented alongside the article attached below.

1.Response to comment: The sentence "Since current examination approaches cannot achieve early diagnosis." is incomplete because it begins with since. Language editing required.

Response: Thanks for your kind suggestion. We have revised the sentence as “As one of the most common tumors, gastric cancer (GC) has a high mortality rate, since current examination approaches cannot achieve early diagnosis.”.

2.Response to comment: Title should be changed as suggested.

Response: I am sorry that I have not found the suggested title. We have tried to revised the original title as “Salivary *Fusobacterium nucleatum* serves as a potential diagnostic biomarker for gastric cancer”.

3.Response to comment: The sentence “we wondered if there was a correlation between salivary Fn and GC.” becomes meaningless when the previous sentence says there is an association. So consider rephrasing the rationale of the study. This part is much better written in the CORE TIP.

Response: Very thanks for your kind suggestions. We have revised the corresponding part as “*Fusobacterium nucleatum* (Fn) primarily colonized in the oral cavity, has been reported to be involved in the development of gastrointestinal tumor. Until now, little is known about the relationship between salivary Fn and GC” in the BACKGROUND part of Abstract.

4.Response to comment: Language editing on aim.

Response: We appreciate the reviewer’s valuable comments. The manuscript has received editing service from Scientific Writing Solutions, USA, and the editing certificate is attached. We hope that this revision of writing is acceptable.

5.Response to comment: In the part of results in abstract and in core tip, the sentence “the Fn level in saliva was associated with the TNM stage” requires language editing. The authors probably are hinting at a correlation with the stage. If that is so then that should be mentioned here, as to how it related.

Response: Very thanks the reviewer's kind suggestion, which are very helpful for improving our manuscript. We have revised this part as "The Fn level in saliva was increased with the TNM stage increased".

6.Response to comment: "Gastric cancer (GC) is the most common malignancy in the digestive tract" is a misstatement.

Response: We appreciate the reviewer's valuable comments. We have modified this sentence as follows: "Gastric cancer (GC) is one of the most common malignant tumors".

7.Response to comment: "The stomach is a cystic organ" is a inappropriate statement.

Response: We appreciate the reviewer's valuable comments. We have deleted this statement in the revised manuscript.

8.Response to comment: "Most patients have peritoneal metastasis or liver metastasis" is to be too bold a comment.

Response: We appreciate the reviewer's valuable comments. We have deleted this statement in the revised manuscript.

9.Response to comment: Does gastroscopy figure in the list of investigations in HEALTHY CONTROLS?? If not then how endoscopy was done in them. Were there no ethical issues?

Response: "HEALTHY CONTROLS" mainly come from physical examiners, their gastroscopy results showed normal. All participants have written informed consent. However, our description is not accurate, and we have revised the "healthy controls" as "normal controls". It represents those with normal gastroscopy.

10.Response to comment: For the sub heading “Fn is abundant in the saliva of GC patients” and “Fn promotes GC metastasis by inducing epithelial-mesenchymal transition (EMT)”, write an open ended sub heading, instead of beginning with an inferential statement.

Response: Thanks for your kind suggestion. We have revised as “The abundance of Fn in the saliva of GC patients” and “The role of Fn in GC metastasis” according to your suggestion.

11.Response to comment: For the sentence “Fn level was significantly higher in GC patients compared with AG, NAG, GP patients and HCs.” how was the significance measured?

Response: Kruskal-Wallis test was performed for global comparison of Fn DNA among GC, AG, NAG, GP patients and NC, and further post-hoc multiple comparisons were used the Mann-Whitney U test. We have added the corresponding part in the “Statistical analysis” part and “figure legends” part in the revised manuscript.

12.Response to comment: Why is the data for Stage IV not mentioned?

Response: All subjects enrolled in this study were come from those who underwent gastroscopy. And, few GC patients with TNM IV underwent gastroscopy. They were mostly diagnosed by imaging rather than pathological result. Therefore, patients with Stage IV were not found in our subjects. To make it clearer, we have made some modifications in the “Study population & sample collection” part.

13.Response to comment: Mention the reference to this statement “GC is the most common tumor of the digestive system”.

Response: This is a misstatement. We have revised the statement as “GC is one of the most common tumors of the digestive system”. Apologize for my negligence.

14. Response to comment: The meaning of “*H. pylori* is the first to think about as a star bacterium.” is unclear.

Response: I'm very sorry for my inaccurate description. We changed it into “*H. pylori* is a well-known risk factor for GC”.

15. Response to comment: Which special structure the authors are referring to in the sentence of “people would prefer to direct colonization due to the special structure of the oral cavity and the gastrointestinal tract”.

Response: We appreciate the reviewer's valuable comments. We have deleted this statement in the revised manuscript.

16. Response to comment: For the sentence: The existing data have shown that the abundance of Fn in GC tissue exhibits a diagnostic power<sup>[15]</sup>, and our experimental data suggested that Fn abundance in the oral cavity was correlated with GC<sup>[15]</sup>. Why is another study being referenced when the authors are talking of their own study results?

Response: I'm very sorry for my mistake. The reference is cited for the front part of the sentence. We have deleted the inappropriate reference in the revised manuscript.

## **Reviewer #2:**

1. Response to comment: It is a good study, but it needs to be studied with more patients for a definite conclusion.

Response: Many thanks for your approval and comment, and we fully accepted the comments. In follow-up studies, we will recruit more cases from multicenter to validate the diagnostic performance of salivary Fn in gastric cancer. Thus, it might be better used in clinics. And we have added these limitations in the revised manuscript.

**Reviewer #3:**

1. The manuscript entitled "Salivary *Fusobacterium nucleatum* serves as a potential biomarker and plays malignant biological role in gastric cancer" by Wen-Dan Chen, Xin Zhang, Meng-Jiao Zhang, Ya-Ping Zhang, Zi-Qi Shang, Yi-Wei Xin, Yi Zhang, presents evidence that Fn abundance in saliva could be used as a promising biomarker to diagnose gastric cancer (GC). Moreover, the Authors suggest that Fn infection could promote GC metastasis by accelerating the EMT process. In particular, the Authors demonstrated Fn abundance in saliva by digital droplet polymerase chain reaction (ddPCR), and established a new simple and effective diagnostic approach to improve the early diagnosis rate of GC. • ddPCR results showed that among the patients with GC and benign gastric disease, and HCs, the Fn level was significantly higher in GC patients compared with atrophic gastritis (AG), non-atrophic gastritis (NAG), gastric polyps (GP) patients and healthy controls (HCs) while there was no difference among AG, NAG, GP patients and HCs; • Moreover, the Fn level was increased with the TNM stage; the Fn level in GC patients with lymph node metastasis was significantly higher compared with those without lymph node metastasis ( $p < 0.001$ ); • The Fn level was significantly higher in GC patients compared with AG, NAG, GP patients and HCs, while CEA, CA199, CA724 and ferritin did not significantly different between GC patients and the other four groups. • The effects of Fn infection in vitro was investigated in infected BGC823 and SGC7901 cells with Fn by the transwell assay and wound-healing assay in the absence or presence of the infected cells. The results obtained in transwell assay indicated that Fn infection significantly enhanced the invasive and migratory capacities of BGC823 and SGC7901 cells; • These results were confirmed by the wound-healing assay. Since EMT is an important process of metastasis, the Authors examined the impact of Fn infection on the expressions of proteins involved in the EMT process by Western blotting analysis. The results revealed that Fn infection significantly decreased the expressions of epithelial

markers, such as E-cadherin, while it increased the expressions of mesenchymal phenotype-associated molecules, such as N-cadherin, vimentin and Snail. This is a novel topic that will be of interest to the readers of the journal. Moreover, conclusions are supported by an appropriate number of evidence.

Response: [Very thanks for your careful comments.](#)

2. Response to comment: Providing evidence (for example through STR DNA profiling) that the two cell lines - BGC823 and SGC7901 - were used in the in vitro experiments are derived from gastric adenocarcinoma.

Response: [BGC823 and SGC7901 are two of the most commonly used cells in gastric cancer study, and here is some examples in 2021\[1-3\]. However, the two cell lines have been suspected of being contaminated with Hela cells. To dispel the suspicion raised by cell contamination, we selected another two GC cell lines \(AGS and MKN-28\) for the experiments. The STR DNA profiling of the two cells AGS and MKN-28 have been provided in the supplementary material. We found that the results obtained from AGS and MKN-28 were consistent with those from BGC823 and SGC7901. We have substituted the figures with the experiment results conducted with AGS and MKN-28 in the revised manuscript.](#)

[\[1\] Sun Y, Xie Y, Tang H, Ren Z, Luan X, Zhang Y, Zhu M, Lv Z, Bao H, Li Y, Liu R, Shen Y, Zheng Y, Pei J. In vitro and in vivo Evaluation of a Novel Estrogen-Targeted PEGylated Oxaliplatin Liposome for Gastric Cancer. Int J Nanomedicine 2021; 16: 8279-8303 \[PMID: 34992365 DOI: 10.2147/IJN.S340180\]](#)

[\[2\] Cui HY, Rong JS, Chen J, Guo J, Zhu JQ, Ruan M, Zuo RR, Zhang SS, Qi JM, Zhang BH. Exosomal microRNA-588 from M2 polarized macrophages contributes to cisplatin resistance of gastric cancer cells. World J Gastroenterol 2021; 27: 6079-6092 \[PMID: 34629821 DOI: 10.3748/wjg.v27.i36.6079\]](#)

[3] Chen W, Zhou Y, Wu G, Sun P. CCNI2 promotes the progression of human gastric cancer through HDGF. *Cancer Cell Int* 2021; 21: 661 [PMID: 34895232 DOI: 10.1186/s12935-021-02352-6]

3.Response to comment: Describing in the introduction the role of *F. nucleatum* in determining an imbalance in the commensal bacterial composition of oral cavity. In fact, it is known that Fn is among the pathobionts that outgrow during dysbiosis preceding periodontal disease (see Nozawa et al., 2020).

Response: Much thanks for your suggestion. *F. nucleatum* (Fn) is a Gram-negative anaerobic bacterium, which is a normal composition of the oral microenvironment. In recent years, due to its increased detection rate in oral infectious diseases, it has been identified as a opportunistic pathogen<sup>[1]</sup>. Chronic periodontitis is caused by the ecological imbalance of the subgingival plaque biofilm communities, leading to the growth of dominant species, which destroys the host immune response, and leads to inflammation. Fn has been proved to be one of the pathogens that grow abnormally before periodontal disease, leading to an imbalance in the composition of oral symbiotic bacteria, which leads to the occurrence of periodontitis. It forms a bridge between more symbiotic early colonizers and more pathogenic late colonizers.

Text has been added to the introduction to clarify the significance role of Fn in determining an imbalance in the commensal bacterial composition of oral cavity.

4.Response to comment: Explaining why they used an MOI of 100 in the transwell and wound healing assays.

Response: The MOI of Fn varied from 10 to 500 for in-vitro experiments in previous studies [1-7]. In our preliminary experiments, we have tried several MOI values, such as 50, 100, 300 and 500. And our data showed similar results



in transwell assays. However, when the MOI was set as 300 and 500, the cells were not able to adhere to the plate in a long time, so that it was impossible to carry out wound healing assay. Therefore, we have used MOI of 100 for our experiments. Moreover, MOI of 100 was more common in the published articles[5-7].

[1] Hirschfeld J, Howait M, Movila A, Parcina M, Bekerredjian-Ding I, Deschner J, Jepsen S, Kawai T. Assessment of the involvement of the macrophage migration inhibitory factor-glucocorticoid regulatory dyad in the expression of matrix metalloproteinase-2 during periodontitis. *Eur J Oral Sci* 2017; 125: 345-354 [PMID: 28776753 DOI: 10.1111/eos.12363]

[2] Casasanta MA, Yoo CC, Udayasuryan B, Sanders BE, Umana A, Zhang Y, Peng H, Duncan AJ, Wang Y, Li L, Verbridge SS, Slade DJ. *Fusobacterium nucleatum* host-cell binding and invasion induces IL-8 and CXCL1 secretion that drives colorectal cancer cell migration. *Sci Signal* 2020; 13 [PMID: 32694172 DOI: 10.1126/scisignal.aba9157]

[3] Harrandah AM, Chukkapalli SS, Bhattacharyya I, Progulske-Fox A, Chan EKL. *Fusobacteria* modulate oral carcinogenesis and promote cancer progression. *J Oral Microbiol* 2020; 13: 1849493 [PMID: 33391626 DOI: 10.1080/20002297.2020.1849493]

[4] Jung YJ, Jun HK, Choi BK. *Porphyromonas gingivalis* suppresses invasion of *Fusobacterium nucleatum* into gingival epithelial cells. *J Oral Microbiol* 2017; 9: 1320193 [PMID: 28748028 DOI: 10.1080/20002297.2017.1320193]

[5] Kang W, Jia Z, Tang D, Zhao X, Shi J, Jia Q, He K, Feng Q. Time-Course Transcriptome Analysis for Drug Repositioning in *Fusobacterium nucleatum*-Infected Human Gingival Fibroblasts. *Front Cell Dev Biol* 2019; 7: 204 [PMID: 31608279 DOI: 10.3389/fcell.2019.00204]

[6] Zhang S, Li C, Liu J, Geng F, Shi X, Li Q, Lu Z, Pan Y. *Fusobacterium nucleatum* promotes epithelial-mesenchymal transition through regulation of the lncRNA MIR4435-2HG/miR-296-5p/Akt2/SNAI1 signaling pathway.

FEBS J 2020; 287: 4032-4047 [PMID: 31997506 DOI: 10.1111/febs.15233]

[7] Chen Y, Chen Y, Zhang J, Cao P, Su W, Deng Y, Zhan N, Fu X, Huang Y, Dong W. *Fusobacterium nucleatum* Promotes Metastasis in Colorectal Cancer by Activating Autophagy Signaling via the Upregulation of CARD3 Expression. *Theranostics* 2020; 10: 323-339 [PMID: 31903123 DOI: 10.7150/thno.38870]

5. Minor comments THE CONTENT OF SOME SECTIONS MUST BE IMPROVED Multiple parts of this manuscript are poorly written. Word vocabulary and grammar need to be improved. Some examples: -page 3, Abstract section, Background “As one of the most common tumors, gastric cancer (GC) has a high mortality rate. Since current examination approaches cannot achieve early diagnosis.” CHANGE as follows: “As one of the most common tumors, gastric cancer (GC) has a high mortality rate, since current examination approaches cannot achieve early diagnosis”; -Write *Fusobacterium nucleatum* as well as *Helicobacter pylori* in Italics throughout the text: i.e. *Fusobacterium nucleatum*. -page 4, Results section “Importantly, the Western blotting analysis further presented that Fn infection significantly decreased the expression of E-cadherin and increased the expressions of N-cadherin, vimentin and Snail.” CHANGE TO “Importantly, the Western blotting analysis further showed that Fn infection significantly decreased the expression of E-cadherin and increased the expressions of N-cadherin, vimentin and Snail.”. -page 4, Core tip “Further cell experiments revealed that Fn could promote the migration and invasion of GC cells by promoting the EMT process” CHANGE TO “Further, experiments in vitro revealed that Fn could promote the migration and invasion of GC cells by promoting the EMT process”. -page 6, a reference to the sentence “*Fusobacterium nucleatum* (Fn) is a Gram-negative anaerobic bacterium, which is essential for the normal oral microenvironment” is lacking. Please, add a reference consistently. -page 7: CHANGE Methods and Materials TO Methods and Materials. -page 7: please

add the code number of Ethics Committee approval. -page 8: complete the sentence “Briefly, after an initial enzyme activation step at 50°C for 10 min and then at 90°C for 10 min, the amplification .....???? were carried out”. -page 9: CHANGE “the cells suspension” TO “the cell suspensions”. -page 13: CHANGE CA199 to CA19-9 and CA724 TO CA72-4. -page 17: add legend to the figure of Western blot assay and CHANGE E-cadheine TO E cadherin. -page 20: please, cut the sentence “EMT is a special program that enables settled epithelial cells to gain the ability to migrate as single cells, which can enhance mobility, invasion, and resistance to apoptosis, conferring metastatic properties of cancer cells[26].” and paste it after the sentence “EMT is a classical pathway promoting metastasis.”.

Response: Thank you very much for your patience in pointing out my mistakes on vocabulary and grammar. We have revised the proposed errors and reviewed the whole article again. And a complete revision of the English language is done by native speaker. We hope all the mistakes on vocabulary and grammar are solved along with the problems in the punctuation.

#### **Reviewer #4:**

1. In this study, Chen WD et al. reported close associations of salivary fusobacterium nucleatum (Fn) with gastric cancer (GC) and its progression. In addition, the demonstrated that Fn enhances motility and invasiveness of GC cells and is also implicated in epithelial-mesenchymal transition of GC cells. This study is well designed, methodology is appropriate, results are beautiful, and discussion is valid. I think that this manuscript is worth publishing.

Response: Very thanks for your valuable comments.

2. Response to comment: Most patients have peritoneal metastasis or liver metastasis when diagnosed: I think that this description is out-of-date and that fewer GC would be found in such an advanced stage today. Please search the literature well.

Response: We appreciate the reviewer's kind suggestions. We have revised the corresponding part in the "INTRODUCTION" part of revised manuscript.

3.Response to comment: (p.10. 1.16) median and range: Is this range SD, SEM, or interquartile range? Please specify.

Response: We are very sorry for our negligence. The range is interquartile range, and we have specified it in the revised manuscript (p.10. 1.13).

4.Response to comment: What is the difference between migration and invasion investigated by Transwell assay? If migration assay is different from invasion, please describe the experimental procedure in the corresponding section.

Response: I apologize for our unclear description. Transwell chambers used for invasion assay were precoated with Matrigel (Corning), while the chambers used for migration assay were not treated. This is the only difference between them, and other experimental steps are the same. And, we have revised the original description.

5.Response to comment: My largest concern is whether or not *H. pylori* may be a confounding factor between GC and Fn infection. Poor hygiene status would be associated with both Fn and *H. pylori* infection. In fact, Fn DNA levels were high not only in GC but in atrophic gastritis that is closely associated with *H. pylori* infection. Meanwhile, *H. pylori* may become underpresented in completed atrophic gastritis, which is an origin of intestinal-type GC. How about the possibility that *H. pylori* hidden behind Fn is actually the cause of GC?

Response: *H. pylori* is a well-known major risk factor for GC, but there is only 1-3% *H. pylori* infections will develop to GC[1]. GC tumorigenesis is involved of a multifactorial etiology, such as diet, obesity, Epstein Barr virus (EBV), genetic predisposition[2]. Usually, pathogens, including Fn, can not colonize

the rest of the gastric microenvironment. Some studies have suggested *H. pylori* infection and colonization might create a suitable microenvironment and likely allow Fn invasion[3-4]. Thus, Fn cooperates with *H. pylori* promote gastric cancer development. Hsieh et al[5]. have found Fn is biomarker for poorer survival of GC patients with *H. pylori* infection. We have added the corresponding part in the revised manuscript.

- [1] Busada JT, Ramamoorthy S, Cain DW, Xu X, Cook DN, Cidlowski JA. Endogenous glucocorticoids prevent gastric metaplasia by suppressing spontaneous inflammation. J Clin Invest 2019; 129: 1345-1358 [PMID: 30652972 DOI: 10.1172/JCI123233]
- [2] Sexton R, Mahdi Z, Chaudhury R, Beydoun R, Aboukameel A, Khan HY, Baloglu E, Senapedis W, Landesman Y, Tesfaye A, Kim S, Philip PA, Azmi AS. Targeting Nuclear Exporter Protein XPO1/CRM1 in Gastric Cancer. Int J Mol Sci 2019; 20 [PMID: 31569391 DOI: 10.3390/ijms20194826]
- [3] Bravo D, Hoare A, Soto C, Valenzuela MA, Quest AF. Helicobacter pylori in human health and disease: Mechanisms for local gastric and systemic effects. World J Gastroenterol 2018; 24: 3071-3089 [PMID: 30065554 DOI: 10.3748/wjg.v24.i28.3071]
- [4] Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, Wu WK, Wong SH, Chen Z, Sung JY, Yu J. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018; 67: 1024-1032 [PMID: 28765474 DOI: 10.1136/gutjnl-2017-314281]
- [5] Hsieh YY, Tung SY, Pan HY, Chang TS, Wei KL, Chen WM, Deng YF, Lu CK, Lai YH, Wu CS, Li C. Fusobacterium nucleatum colonization is associated with decreased survival of helicobacter pylori-positive gastric cancer patients. World J Gastroenterol 2021; 27: 7311-7323 [PMID: 34876791 DOI: 10.3748/wjg.v27.i42.7311]

**Company editor-in-chief:**

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Response: Very thanks for your kind suggestion, which are very helpful for improving our manuscript. We have revised the format of the Figures and Tables, as well as the Figure legend according to the suggestions and guidelines, expecting to meet the requirements of the magazine. Thanks again. The manuscript also has been edited by Scientific Writing Solutions, USA for grammar. These changes are marked in red and they will not influence the content and framework of the paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

**Re-reviewer:**

1. I appreciated the efforts of the Authors in revising this manuscript. The manuscript has been improved as compared to the previous version.

Response: Much thanks for your patient reviews and your recognition of my

work.

2.The Authors answered to have added a text on the role of Fn in the Introduction (see the attached answer to point 3). However, I did not find it. Please, be consistent. Moreover, I found a typo: CHANGE a opportunistic to an opportunistic.

3.Response to comment: Describing in the introduction the role of *F. nucleatum* in determining an imbalance in the commensal bacterial composition of oral cavity. In fact, it is known that Fn is among the pathobionts that outgrow during dysbiosis preceding periodontal disease (see Nozawa et al., 2020).

Response: Much thanks for your suggestion. *F. nucleatum* (Fn) is a Gram-negative anaerobic bacterium, which is a normal composition of the oral microenvironment. In recent years, due to its increased detection rate in oral infectious diseases, it has been identified as a opportunistic pathogen<sup>[1]</sup>. Chronic periodontitis is caused by the ecological imbalance of the subgingival plaque biofilm communities, leading to the growth of dominant species, which destroys the host immune response, and leads to inflammation. Fn has been proved to be one of the pathogens that grow abnormally before periodontal disease, leading to an imbalance in the composition of oral symbiotic bacteria, which leads to the occurrence of periodontitis. It forms a bridge between more symbiotic early colonizers and more pathogenic late colonizers.

Text has been added to the introduction to clarify the significance role of Fn in determining an imbalance in the commensal bacterial composition of oral cavity.

Response: I am sorry that this answer is not quite consistent with the corresponding part of the article. We have modified it in the revised

manuscript according to the content in the previous reply, the content is as follows:

*Fusobacterium nucleatum* (Fn) is a Gram-negative anaerobic bacterium, which is a normal composition of the oral microenvironment. In recent years, due to its increased detection rate in oral infectious diseases, it has been identified as an opportunistic pathogen<sup>[1]</sup>. Chronic periodontitis is caused by the ecological imbalance of the subgingival plaque biofilm communities, leading to the growth of dominant species, which destroys the host immune response, and leads to inflammation. Fn has been proved to be one of the pathogens that grow abnormally before periodontal disease, leading to an imbalance in the composition of oral symbiotic bacteria, which leads to the occurrence of periodontitis. It forms a bridge between the early symbiotic colonizers and the late pathogenic colonizers.

Sorry again for the typo. It has been identified as an opportunistic pathogen.

3. In the answer to point 4 the Authors explain why they used MOI 100 in their experiments. Please, the Authors have to add also that this value was common in the published articles.

Response: Thank you very much for your suggestion. And we have added this part in the revised manuscript according to your suggestion: The Fn were added to the cells at an MOI of 100 based on the preliminary experiments, and this value was common in the published articles.