Point-to-Point Responses to the Reviewers' Comments

Editor

We sincerely appreciate your comments. We provided point to point answer to the editor and all reviewers. In addition, we added "This is only true if the corrections we made are changed." at the end of #12 section in the 47858-Copyright License Agreement with signatures of authors. Please note we made important corrections.

[1] For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies.
Please provide point to point answer to all reviewers.

Response) This manuscript was copyedited by a native-English speaker (Mark Donowitz). We provided point to point answer to all reviewers. In response to this comment, Mark Donowitz additionally edited the manuscripts. Revised parts are highlighted in the updated version of the manuscript.

[2] You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by...".

Response) We provided the two certificates of funding agency for two grants (No. 2019R1H1A1035601 and No. 2015R1C1A1A02037048). We also added "; the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning, No. (2015R1C1A1A02037048)." Revised parts are highlighted in the updated version of the manuscript.

[3] Please offer the audio core tip, the requirement are as follows:
In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip. This audio file will be published online, along with your article. Please submit audio files according to the following specifications:
Acceptable file formats: .mp3, .wav, or .aiff
Maximum file size: 10 MB
To achieve the best quality, when saving audio files as an mp3, use a setting of 256 kbps or higher for stereo or 128 kbps or higher for mono. Sampling rate should be either 44.1 kHz or 48 kHz. Bit rate should be either 16 or 24 bit. To avoid audible clipping noise, please make sure that audio levels do not exceed 0 dBFS.

Response) We provided the audio core tip (mp3 file).

[4] Please distinguish between the title of the article series. Three levels of subtitles are allowed: (1) First subtitle: All in bold and capital; (2) Second subtitle: All in bold and italic; and (3) Third subtitle: All in bold.

Response) In response to this comment, we changed all the subtitles. Revised parts are highlighted in the updated version of the manuscript.

[5] Please check and confirm that there are no repeated references!

Please add PubMed citation numbers (PMID NOT PMCID) and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.
PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) (Please begin with PMID:)
DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.**)

Response) In response to this comment, we confirmed there are no repeated references and revised the references.

[6] Please provide the decomposable figure of figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. -Figures.ppt" on the system, we need to edit the words in the figures. All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes.

Response) We provided the decomposable figure of figures, whose parts are all movable and editable, organized into a PowerPoint file, and submitted as "47858-Image File.ppt" on the system. All submitted figures, including the text contained within the figures, are editable. We provided the text in the figure(s) in text boxes.

[7] Please don't include abbreviations in the title of the figure/table.

Response) In response to this comment, we changed "IBD" to "Inflammatory bowel diseases". We also changed "IECs" to "intestinal epithelial cells"
Revised parts are highlighted in the updated version of the manuscript.

Reviewer #1

Dear authors Thank you for the interesting review. Just very few comments.
Dear authors
Thank you for the interesting topic you reviewed.
The subject is interesting, and ideas are clearly expressed, and the manuscript is well organized.

We appreciate your encouragement and positive comments. Revised parts are highlighted.

Few comments:

- Unclear sentence:
  - Page 6, line 3; ”…. human intestinal epithelium even after many ???passages”. What do you mean by passages???

Response)
Passage is a subculture in which cell culture is continued by transferring some or all cells from a previous culture to fresh growth medium.

  - Pages 19-20; It seems weird to culture the enteroids from the diseased/ ulcerated segments of IBD if aiming at therapeutic target???!!!! Why not cultured from healthy integrated cells????

Response)
The studies of IBD enteroids are just starting, so comments may or may not be correct. The intestinal enteroids/organoids derived from the diseased/ulcerated segments of IBD might resemble more closely the inflammatory phenotype of the IECs of IBD and differ from those from the uninvolved segments of IBD tissue. Therefore, generating IBD enteroids from diseased segments rather than uninvolved segments of IBD, might have advantages to understand IBD pathogenesis. Of note, this is just a hypothesis at this time.

- Editing:
  - Spelling mistake, page 11, line 2>>> moderate NOT moderat

Response)
In response to this comment, we changed the word on page 11 “moderat” to “moderate”.

  - Inappropriate word, page 15, 2nd paragraph, 2th line;” …prolonged IFNγ **exposure**…”. It’s better replaced by secretion, release,…etc.

Response)
In this reference (J Exp Med. 2014 Jun 30;211(7):1393-405), IFN-γ was not a secreted protein but was used to stimulate enteroids. To clarify the meaning of the sentence on page 16, we changed the “IFN-γ exposure” to “stimulation with recombinant IFN-γ (1 ng/ml)”.

- General Formatting:
  - Initial tabbing for every newly thematic paragraph.

Response)
We added a tab at the beginning for every newly thematic paragraph.

  - References

Response)
We checked references.

Reviewer #2

The authors have done a good review of the present status of intestinal organoids use in IBD along with possible future applicability and limitations. Only two more features may be further discussed.

We appreciate your helpful comments. Revised parts are highlighted.

[1] The use of organoid cultures for high-throughput drug screening, genetic editing and gene silencing etc as may be applicable for IBD treatment.

Response)
In response to this comment, we added several sentences and paragraphs on page 6-7 "In particular, growing enteroids as polarized monolayers instead of spheroids allows direct apical and basolateral access by pathogens and oral drugs, and subsequently enables the effective study of ion transport and secretory functions. A recent study demonstrated the successful use of enteroid monolayers in drug discovery by miniaturizing mouse colonoid monolayer cultures to 96-well plates, and conducting a phenotypic screen of approximately 2,000 drug candidates[44]. We have adopted the following approach for development of anti-diarrheal drugs. Identification of drug targets includes studies in diarrheal models in human enteroid monolayers. Initial drug candidates are screened early for toxicity in human enteroids with further development curtailed if human intestinal toxicity is identified. Once pharmacokinetic approaches are carried out in mouse intestine and human colon cancer cell lines, human enteroids are studied to determine IC50 and if similar it is considered that the specific drug can be further developed. This approach was used with the CFTR inhibitor BPO-27 which is now under development by pharma for phase I and II studies[45].

Human enteroids are amenable to lipofectamine-, low voltage electroporation-, and viral-based genetic manipulation including knock down, knock-out, knock-in, or overexpression[38]. The CRISPR/Cas9 system was also used to edit the genome of intestinal enteroids derived from cystic fibrosis patients and repaired the cystic fibrosis transmembrane conductance regulator (CFTR) function[46]."

[2] The use of organoids from very young patients having rare forms of IBD [e.g. very early onset IBD caused by monogenetic aberrations like IL-10, IL-10R, XIAP, NCF2, or TTC7] may help to understand the role of those genes and lead to drug discovery that reverse the consequences of those mutations. [e.g. intestinal organoids grown from children with multiple intestinal atresia showed an inverted growth with the cells apical side on the outside of the organoids; treatment with a Rho kinase inhibitor led to reversal of this inversion and could help in the development of new therapies for this condition

Response)

In response to this comment, we added a paragraph on page 15 “Rare subtypes of IBD with very early-onset in early childhood (VEO-IBD) are more likely to be associated with specific genetic mutations, especially associated with immune dysfunction, including in IL-10, IL-10R, XIAP, NCF2, or TTC7. These do not occur in IBD in older children[136]. Using enteroids derived from these young patients can lead to the discovery of compounds which are able to normalize the consequence of those mutations. For example, intestinal enteroids grown from children with multiple intestinal atresia, a rare congenital disease with IBD-like features by TTC7A mutations, displayed an inversion of apical-basal polarity of the epithelial cells that was reversed by Rho kinase inhibitor[137]. More recently, whereas TRAIL stimulation induced caspase-3 cleavage and cell death in intestinal enteroids derived from healthy donors, caspase-8 deficient intestinal enteroids derived from a VEO-IBD patient were unresponsive to TRAIL, revealing caspase-8 deficiency as a novel cause for VEO-IBD associated with defective epithelial cell death response[138].”

[3] Under limitations discuss also the difficulties in development of a reliable model of microbiota–epithelium interactions.

Response)
In response to this comment, we added a paragraph on page 20-21 “Considering the recent progress in establishing human enteroids as a model of infectious diarrheal diseases, human enteroid/organoid culture conditions might provide a relevant pathophysiologic model to study microbiota-epithelium interactions[38,146]. However, to develop a more reliable model, ways to co-culture the primarily anaerobic gut microbiota, which consists of many bacterial species, with human enteroids in anerobic culture conditions without damaging the enteroids is a significant challenge to be overcome[9]. Moreover, intestinal enteroid/organoid culture systems lack physiological luminal and blood flow and the repetitive contractions of peristalsis, which are known to result in altered gene expression and cell function/morphology[168]. In this regard, an emerging microfluidic chamber system combining peristalsis-like stretch with apical and basolateral liquid flow (gut-on-a-chip) might be a way to overcome these drawbacks of enteroid/organoid cultures. Most recently, a novel microfluidic device with a transluminal hypoxia gradient was developed for co-culturing a complex living human gut microbiome, including obligate anaerobes with human intestinal epithelium, that until now has only included Caco-2 cells[169].”

Reviewer #3

These authors have done a splendid job of reviewing and elucidating the means by which in vitro techniques can be exploited to explore the therapeutic potential of various agents in IBD as well as other diseases.

We appreciate your helpful comments. Revised parts are highlighted.

To round out their extensive review of intestinal organoids as a research tools, the authors might want to give at least a brief mention to the current and past use of human fetal intestinal organ

culture (e.g., Proc Natl Acad Sci USA 2000;97:6043-8. J Exp Med 1988;167:1341-9. Pediatr Res 2015;77:528-35).

Response)
In response to this comment, we added words on page 5 "human fetal intestinal organ cultures" and a paragraph on page 6 "Human fetal intestinal organ culture, which is prepared from intestinal tissue obtained from therapeutic abortions, has been used to study the fetal intestinal immune response to luminal microbes[39] and the pathogenesis of necrotizing enterocolitis[40] and celiac disease[41]. Human fetal intestinal organ culture has a strength of providing multiple cell types and sequential differentiation which are not preserved in intestinal enteroid/organoid cultures. However, in comparison to enteroids/organoids, intestinal human fetal intestinal organ culture has several weaknesses. These include the application of high throughput drug screening, difficulty in obtaining fetal tissue, short viability (up to 48h), limitation in delivery of exogenous stimuli and real-time monitoring due to the thickness of the intestinal tissue[42]."

On the other hand, given the laboratory-oriented focus of this technical review, it might not be necessary to devote quite so much space and bibliography to articles regarding current clinical therapeutic approaches to IBD.

Response)
We respectfully disagree. One of our goals is to review the current state of use for IBD studies and to encourage further studies in this area.

Reviewer #4

Yoo JH et al. (Manuscript Number: 47858) perform review on "Intestinal enteroids/organoids: a novel platform for drug discovery in inflammatory bowel diseases". However, I have following concerns about this work at least in its present form.

We appreciate your helpful comments.

1. This manuscript must be copyedited by native-English speakers before resubmit it.

Response) This manuscript was copyedited by a native-English speaker (Mark Donowitz).

2. It is recommended to rewrite the abstract and full text according to the formats of "introduction, materials and methods, results, discussion and conclusions".

Response) This manuscript is an invited review article (not original article), therefore, this manuscript fits the journal's format for review article (introduction, main text, conclusion).

3. Please delete the redundancy sentences, and use the tables or figures to show information.

Response) We have reduced the redundancy, and used the tables and figures to show information.

4. On the section of Discussion, the main findings, limitations, and authors' recommendations should be present more clearly and comprehensively.

Response) This manuscript is an invited review article (not original article), therefore, this manuscript does not contain a discussion section. However, in the main text, we described the limitations of intestinal enteroids as a research tool for IBD using the authors' comments.

5. This manuscript must fit the journal's requirements or format.

Response) This manuscript fits the journal's all requirements and format.

Reviewer #5

I would like to congratulate you for this excellent review.

Response) We really appreciate all your encouragement and positive comments.

Reviewer #6

Dear Authors, Congratulations for this extensive review. Topic is new and relevant to current research; review is nearly perfect, although it is pertaining to basic science. There is no window left for me to suggest any changes. All the best.

Response) We really appreciate all your encouragement and positive comments.