

“Developing Captive Marmosets as a Translational Geroscience Model to Study the Microbiome and Healthy Aging”

Reviewer #2

#	Comments	Authors' Response	Manuscript Changes
1	The title of the review does not correspond to its content. The review contains information that, from my point of view, is not very well structured, and in principle is far from the “developing captivity of the monkey”. The authors have mixed everything up. The text focuses too much on <i>Clostridioides difficile</i> infection, which is not correlated with age. This is an infection, as well as <i>Salmonella</i>, <i>Shigella</i>, <i>Klebsiella</i>! The appearance of cells in the intestine does not correlate with age. So, it is necessary to deeply separate the sources of dysbiosis appearance in old humans.	We apologize for the confusion. Please see responses below. Title: the article describes the use of marmosets housed in captivity to study the microbiome and aging, rather than developing captivity. To reduce confusion, we have changed the title to remove “captive.” Relationship to <i>C. difficile</i>: We included a single paragraph on <i>C. difficile</i> infection with the purpose of introducing fecal microbiota transplantation as a potential intervention in aging. <i>C. difficile</i> infection is the therapeutic space where FMT has traditionally been used and where science has demonstrated the ability of FMT to significantly alter and restore the microbiome to improve health in these patients. Inclusions of methods used to develop FMT for <i>C. difficile</i> are relevant to the proposed new protocol as well. We also include a brief description of new <i>C. difficile</i> microbiome-targeted therapies to support that access to these therapies has increased in recent years, which will aid in translation to other therapeutic spaces (e.g., aging). Lastly, <i>C. difficile</i> infection is highly correlated with age. Approximately 60% of <i>C. difficile</i> infections occur in adults over the age of 65 years and older adults are at significantly greater risk for poor outcomes, as are older adults with most other health conditions. Thus, we feel that our brief inclusion of <i>C. difficile</i> is well-justified.	Title: Developing the Common Marmoset as a Translational Geroscience Model to Study the Microbiome and Healthy Aging

2	Several aspects of FMT are discussed in a row in the text: sources of stool, methods of sample preparation, methods of administration, methods of storage, etc. Everything in a row, without accents and conclusions in the text as I should be in the Review. The text provides detailed methods of how and what was mixed for FMT, but the Review should contain generalizations explaining the purpose of these details. Moreover, it follows from the text that it is extremely difficult to cultivate and store cells for FMT, which, of course, will lead to negative or unpredictable results. Conclusions should be drawn at the end of each subsection based on the real (positive and negative) information provided.	We have included clearer conclusions for each section as suggested. Please see the first sentence of each main protocol paragraph for main takeaways. We have also added in limitations to the conclusion sections.	Please see protocol revisions throughout. Conclusions, lines 373-376: The marmoset FMT protocol herein was developed based on critical review of human, rodent, and primate FMT literature. Given the many complex steps for acquiring material, processing, and administering FMT, the protocol necessitates validation for scientific and therapeutic purposes.
3	I recommend to introduce the Table 1A from the Appendix A as Table 2 to the main text of the paper (Line 192). In this case, the numeration of references in this Table should be corrected.	Given that the appendix has a large amount of data that was used to inform the marmoset FMT protocol, but that include many studies indirectly related (e.g., mouse studies), we feel that the appendix may be the more appropriate positioning within the article; however, we will defer to the editor for final decision. Of note, we have renamed Table 2 as there was an original typo in the table label.	Please see revised Table 2 title.
4	The change in concentrations of hormones regulating the presence of glycogen in the intestine walls is one of the main problems of dysbiosis with increased age. The probiotics, used the receptors associated with glycogen, lost their chance to be well attached to the intestine walls. After that, it is possible to introduce the probiotics by different ways (with or without antibiotics, orally or via gavage, etc.), the result will not be long and efficient. This information should be disclosed in the review, because it is important.	We have included an additional statement in the introduction to address this.	Lines 40-43: Poor functional changes include a reduction in important microbial metabolic byproducts, such as short-chain fatty acids, vitamins and minerals, and energy-storing glycogen [2,3] and a reduction in pathogen protection via production of antimicrobial molecules by commensal microbes [1].
5	Please add the reference for the data performed in Table 1.	References to support each step in the procedure are included throughout the	Please see references throughout the “Marmoset FMT Protocol” section.

		supporting paragraphs below the table. We feel that adding in all references within the table would be redundant, but we will defer to the Editor for guidance.	
6	Line 232: This is Table 2, but not Table 1. The appearance of the compiled information in the Table 2 should be explained and the references for the information should be mentioned. In case the development of these steps was made by the authors, the paper converts to the experimental paper instead of Review! In addition, there are many places in the article where the authors describe their experiments (line 103 - we propose to use a nonhuman primate model, line 132 - we developed for studying FMT, line 148-149 - We first conducted a cross-sectional study, line 152 - we found, line 158-159 - we conducted a prospective study, line 164 - we did note significant differences, lines 172-173 - We designed the protocol, line 174 - We also chose to use oral administration, line 176 - we believe this protocol may be used, lines 183-184 - we included health span measures, line 193 - We designed the protocol to specifically study, line 194 - We also chose to use oral administration, lines 240-241 - we chose to collect enough stool, line 242 - we needed a total of 78 doses, line 243 - we needed a minimum of 19.5g stool, line 244 - we collected a total of 50g stool, line 256 - we recommend freezing samples, line 269 - e prepared samples in an anaerobic chamber, line 294 - we noted some shift in microbiome composition, lines 355-356 - we enrolled geriatric marmosets), which classifies this article as experimental! And in this case, the material of the article should be presented in accordance with another template (with materials and methods). Thus, as a Review such paper should be rejected.	All of the examples provided by the reviewer refer to previously published work or new protocols in which data have not yet been published. No new data are presented in the current manuscript. We have reviewed the full literature review and protocol sections to ensure that this is clear.	Please see minor revisions throughout the literature review and protocol sections. Specifically, we removed the word “we” as much as possible, as well as unpublished work.

7	Please, see the Line 206 (<i>Faecalibacterium</i> , <i>Eubacterium</i> , <i>Clostridium</i>), lines 154,156 (<i>Firmicutes</i>), lines 48, 155,156 (<i>Proteobacteria</i>), lines 178,198,212,259 (<i>C. difficile</i>), line 207 (<i>Roseburia</i>), line 209 (<i>Enterobacteriaceae</i>), etc. All names of the microorganisms should be italicized. I recommend to check this carefully.	We have revised the manuscript such that any organism in which the genus or species is discussed has been italicized. Bacterial phyla are usually not italicized.	Please see new italicized organisms throughout the manuscript.
8	Finally. The authors analyze mice as a model on which an oral method of fecal administration to sick animals was performed. Do the authors of this article really suggest using the same FMT method of stool administration (ingestion of human feces as part of food) for humans after all?	No, we do not. Oral administration via food or oral gavage is often necessary in animals as other procedures typically conducted in humans (e.g., enema or colonoscopy) may not be feasible. We have now included this as a limitation of using marmosets for human translation.	Lines 322-328: Oral gavage dosing was chosen primarily for feasibility and animal safety. Oral is also the primary delivery route used in rodent FMT models. It is possible that oral delivery could result in loss of donor microbes as they transit the more acidic upper gastrointestinal tract. In humans, oral delivery is done using large, enteric coated capsules that are swallowed, which is infeasible for animals. Additional studies of lower gastrointestinal FMT delivery (e.g., enema) on microbiome structure, function, safety, and efficacy are needed, as lower delivery is common in humans.