Response to Specific Reviewer Comments (Manuscript No. 24321)

Reviewer 1 Comments:	**Our Response:**
The review entitled Chitosan nanoparticles for oral gene delivery represents an accurate and up to date assessment of the field.	We appreciate the reviewer's evaluation of the manuscript and hope that the concerns have been addressed below.
This manuscript describes the use of chitosan as a DNA gene carrier for oral gene delivery.	We hoped to present this in the context of the barriers that a chitosan-DNA complex faces in a host.
Generally, the review has been described in a good manner.	Thank you for your feedback. We have worked to address your concerns below.
However, the review is similar to many others than have been written in this area over the past year.	While oral gene therapy has been explored and chitosan analyzed previously, we were unable to come across a review that had a thorough compilation of the numerous host-related barriers that a vehicle (i.e. chitosan), and the vector have to overcome in order to show efficacy. We believe that having a review such as ours will provide compiled information for the readership of the journal, and highlighting key natural hurdles gene therapy faces.
Several modifications of text need to be performed before this manuscript will be accepted.	We have used the reviewer's below suggestions to make these modifications. Additionally we made additional revisions to the entire manuscript.
I suggest that authors to reorganize the abstract.	This has been addressed, specifically as suggested below.
The description of ' A good example of this is the hype felt in 1989 after the cloning of the cystic fibrosis gene, a very common and devastating genetic disease that destroys lung function. It was assumed that the inhalation of the responsible gene would lead to immediate restoration of lung function and cure' is too much speculation; need to add some references to confirms.	We agree that this statement appears highly speculative and in response to the reviewer's comment we have removed this statement.
Again, abstract talked about unwanted immune response caused by gene deliver. But in the review, the authors wrote more in the field of capacity of chitosan to	We understand the point that the reviewer has made regarding immune response. In order to simplify the

induce desired immune response.	abstract, this section was removed.
In conclusion, the authors wrote 'oral gene therapy has been explored for the treatment of cancer by delivery transgenes that code for 'suicide proteins' in cancer cells'; I did not find any description about this in the text of review.	We understand that this was a new concept introduced in the conclusion. The point of it was to emphasize the vast utilization of gene therapy and its promise. This has been clarified and changed to: "While oral gene therapy has shown immense promise as treatment options in a variety of diseases, there are still significant barriers to overcome before it can be considered for clinical applications."
So the authors should concentrate on the emphasis of extracellular and intracellular barriers of oral gene delivery and strategies how to overcome these barriers by using chitosan and chitosan-modified nanoparticle.	We have changed the abstract to focus on extracellular and intracellular barriers to oral gene delivery: "There are still a number of barriers that chitosan DNA nanoparticles must overcome and that we must better understand in order to make further advancements in oral gene delivery. In this review we provide an overview of the physiologic challenges facing the use of chitosan DNA nanoparticles for oral gene delivery at both the extracellular and intracellular level. From administration at the oral cavity, chitosan nanoparticles must traverse the gastrointestinal tract and protect its DNA contents from significant jumps in pH levels, various intestinal digestive enzymes, thick mucus layers with high turn over, and a proteinacious glycocalyx meshwork. Once these extracellular barriers are overcome, chitosan DNA nanoparticles must enter intestinal cells, escape endolysosomes, and disassociate from genetic material at the appropriate time allowing transport of genetic material into the nucleus to deliver a therapeutic effect. "

By the way, some schema or tables or figures may to add in the text to enforce the author's ideas.	The figure legend have been revised: **Figure 1:** chitin and chitosan molecular structure **Figure 2:** chitosan-DNA nanoparticles and GI tract pH **Figure 3:** GI tract enzymes and Chitosan-EDTA molecule **Figure 4:** Effect of gastrointestinal enzymes on chitosan-DNA nanoparticles **Table 1:** Review of enzyme inhibitors combined with chitosan nanoparticles for drug delivery **Figure 5:** Glycocalyx brush border and nanoparticle size requirements **Figure 6:** proton sponge effect **Figure 7:** Model of DNA combined with importin/NLS complex to enhance importation of DNA through nucleopores.

Reviewer 2 Comments:	**Our Response:**
In this review, the authors discussed the challenges facing oral administration of chitosan DNA nanoparticles, including various extracellular and intracellular barriers of oral gene delivery and how to overcome the hurdles using chitosan and chitosan-modified nanotechnologies.	We appreciate your feedback. Yes, this is an accurate description of our review.
The figure captions should be revised to better explain the main point of each figure	All figure captions have been revised.
The texts in figures are too small to	Text was made larger (size 10 minimum).

read	Images were also made larger to make text more visible.
In Figure 1, the pH in stomach should be 1-2 or 1-3 instead of exactly 1.7	The pH of the stomach was changed from the mean (1.7) to the range (1.4-2.1).
In Figure 2, we can find out the change of nanoparticles' charge in different pH, but it is hard to indicate the stability and size. Getting the stability and size data from references and shown in a table with this figure would be better.	It is difficult to find numerical data on the effect of pH on nanoparticle size (e.g. in nm). The reviewed papers did describe nanoparticle shape, as well as include photos of their video-enhanced fluorescence microscopy techniques. A sample of these images were included and referenced in fig. 2 to demonstrate to the readers the visual change in nanoparticle shape and size with pH changes.
On page 5, line3, combined with a chemical structure of chitosan would be helpful for this description	The pKa of 6.5 was added to a new figure 1 that demonstrates chitosan's molecular structure.
On page 11, line 3, 2-3 original references on proton sponge effect should be given here for better understanding of this theory for readers from different disciplines.	The following references were added: Douglas KL, Piccirillo CA, Tabrizian M. Cel line-dependent internalization pathways and intracellular trafficking determine transfection efficiency of nanoparticle vectors. Eur J Pharm Biopharm. 2008 Mar;68(3):676-87. Richard I, Thibault M, De Crescenzo G, Buschmann MD, Lavertu M. Ionization behavior of chitosan and chitosan-DNA polyplexes indicate that chitosan has a similar capability to induce a proton-sponge effect as PEI. Biomacromolecules. 2013 Jun 10;14(6):1732-40. *Please note, as a result we have adjusted the reference numbers accordingly.
On page 11, line 5, it should be H2O not H20.	H20 was changed to H_2O.

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