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Dear Editor

Please find enclosed the edited manuscript in Word format (file name: 16550-reivised. Doc)

Title: Applications of human hepatitis B virus preS domain in bio- and nanotechnology

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Name of Journal: *World Journal of Gastroenterology*

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Thank you very much for giving us the opportunity to revise our manuscript. We also really appreciate the reviewer's useful suggestions and comments on our manuscript (Manuscript number 16550). We have carefully considered the reviewer's suggestions and comments, and have revised our manuscript.
Our response to the reviewers' comments and suggestions is as follows:

Reviewer 1
Comment 1) This is a good review describing how pre-S HBV domains can be used for drug delivery into hepatocytes. The review is well-written; however, there are some misspellings and the sentences are too long. This should be optimized.

Answer) We have carefully checked and corrected spelling errors. It's the fact that some sentences are long, but we think they can't be hard to understand and connect.

Comment 2) According to summary, pre-S-based particles cannot be used for specific delivery of drugs into hepatocytes. What is known about the mechanisms? Is it because PreS1 binds to ASGP receptor, which might be blocked in inflamed liver?

Answer) Thank you very much for valuable comments. The preS-based delivery system shows high hepatocytic cell-targeting ability and efficient delivery of diagnostic and therapeutic molecules (e.g., drugs, genes, and proteins) into hepatocytic cells, because the preS can specifically recognize and bind to them through target receptors (e.g., ASGP receptor). Although further studies are needed, a recent study suggested that the preS1-fragment (aa 10–36)-fused

virus-like particles can bind strongly to HepG2 cells, but is not transferred into the cytosol. On the other hand, virus-like particles containing preS1 aa 2–108 was endocytosed and was observed in the cytosol. These results may mean that the delivery system conjugated with short preS1 fragments can specifically bind to hepatocytic cells, but has not shown satisfactory transfection efficiency and therapeutic efficacy. However, as mentioned above, further studies are required to define the mechanism.

Reviewer 2

Comment 1) In this review, Dr Toita and colleagues have clearly explained the present knowledge in regards to the application of the HBV preS-domain in diagnostics, therapeutic/vaccine targeting and a hepatocyte-delivery system for diagnostic and therapeutic molecules. The article is well written, concise and informative in all sections. All references are adequate. However, in regards to HBV genotype H (INTRODUCTION), specific citations should be given, due to the fact that this genotype is exclusive to Mexico and it has higher genetic variability than other genotypes. Therefore, the authors should express in the convenient section their opinion about how these bio- and nanotechnology applications could be influenced by the genetic differences in HBV genotypes for this specific region. This review is very interesting, thank you.

Answer) I am very thankful to you for excellent comments. As mentioned in the text, the HBV virus is classified into at least eight genotypes (A–H) which have distinct geographic distributions. The eight HBV genotypes show very similar sequences in essential residues (aa 9–18) within receptor binding sites located in the preS domain (Figure 2). These results, however, may not mean that bio- and nanotechnological approach using a HBV genotype-derived preS domain can be applicable to other HBV genotypes, due to high genetic diversity at the preS domain. According to your comments, the above sentences have been added to SUMMARY AND OVERALL CONCLUSIONS.

Reviewer 3

In this excellent review article Dr. Toita and colleagues summarized and discussed the present knowledge of the application of HBsAg and especially the HBV preS-domain in diagnostics, therapeutic/vaccine targeting and as hepatocyte-delivery system for diagnostic and therapeutic molecules. The article is comprehensive, well and appropriate referenced and yet concise in its content. The introduction introduces the following sections well. The review article is informative and very interesting to read. The scheme of therapeutic and diagnostic options and figure of the S-domain was well constructed, concise, and easy to follow. The main and

important aspect of the current research on the potential application of HBsAg in bio- and nanotechnology has been well addressed. Actually, there is no criticism with regard to the content and sufficiency of the manuscript.

Answer) Thank you very much for your positive evaluation on our manuscript.

All corrections and alterations in the text have been highlighted in red.

We believe the manuscript has been improved satisfactorily and meets criteria for publication in your journal. Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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