Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript "A novel sericin-based hepatocyte serum-free medium and sericin's effect on hepatocyte transcriptome". The comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made corrections which we hope to meet with approval. The main corrections in the paper and the responses to the reviewer's comments are as following:

The response to reviewer1 (00183445)
Advise: It would be good to add what cells are used in "the bioartificial liver support system (BALSS)".
Response: Thank you for your suggestion. At the present time, the cells used in BALSS are mainly primary porcine hepatocytes[1] and immortalized cells, such as HepG2 and C3A[2]. HepG2 was a cell line derived from hepatocellular carcinoma. It has been widely used in the research on BALSS, because of its rapid proliferation and excellent cell function[3]. C3A is a clonal derivative of HepG2, with higher albumin production than HepG2, and C3A cells also exhibit an excellent ability of ammonia elimination. C3A is the hepatocyte used in the ELAD, which has been proved to be effective in liver supporting and biocompatible to patients in clinical trials[4]. (The text above has been added into the second paragraph of introduction.) So C3A was selected as the subject in this study. In the following studies, we will verify whether this serum-free medium is suitable for the other hepatocytes commonly used in BALSS, such as HepG2, HuH7 and primary porcine hepatocytes. Thanks for your valuable advice.

The responses to reviewer2 (00503516)
Comment: The first three lines of M&M should be moved to the beginning of the result section to make clear the general experimental organization.
Response: Yes, we agree with you, it would be clearer to read. However, the guideline for manuscript preparation of BPG recommends that all tables should be presented behind the main text. We are sorry that we have to format the manuscript according to the guideline.

Comment: It would have been better to use more than one hepatic cell line, indeed it is not clear whether the results obtained are of general value or are restricted to the C3A cell line used; in this last case the relevance of the manuscript would be significantly reduced. This aspect should be at least commented in the discussion as a limitation of the study.
Response: Yes, this is one of the limitations of this study. Although it is proved that our serum-free medium is suitable for *in vitro* culture of C3A cells,

and sericin promotes the attachment and proliferation of C3A cells, it is still not clear whether the results obtained are restricted to the C3A cells. In the following study, we are going to verify whether this serum-free medium is suitable for the other hepatocytes, and the effect of sericin on the other hepatocytes, such as HepG2, HuH7 and primary porcine hepatocytes. (This has been added before the last paragraph of the main text.)

Comment: Define in the text of the result section what are treatment groups A, B, C and D, the definition is reported only the figure legend.
Response: In part 1, group A is our novel serum-free medium, group B is HepatoZYME, group C is the complete medium (DMEM/F12 with 100 ml/L FBS), and group D is DMEM/F12. The definition of each group has been added into the beginning of result section (at the first paragraph of result section). Thank you for your advice.

Comment: The data of Fig 1 should be quantified and shown as histograms.
Response: Thank you for your advice. We have added the histogram into Figure 1.

Comment: The most relevant weakness of the manuscript depend on the fact that the authors should have tested the functions of C3A (urea generation, figure 2) under an overload of NH4+ and see the ability of the cells to convert it into urea. Similarly, it would have been interesting to study the ability of C3A to convert an overload of non-conjugated bilirubin into conjugated bilirubin. This is in the light of the fact that BALSS is used for patients with acute liver injury and end-stage liver failure where the NH4+ and non conjugated levels of bilirubin are expected to be considerably augmented. The lack of the above suggested experiments should be at least commented in the discussion as a limitation of the study.
Response: Thank you for your guidance, we recognize these limitations of the study. However, the time for revision is only 7 days, we are afraid that we don't have enough time to do the experiments again. But we really value your opinion, so we will improve our design of experiments in the following studies. The comments about limitations have been added before the last paragraph of the main text.

Comment: In section "Part 2, Live/Dead fluorescence microscopy assay" fig 6 should be substituted by fig 4. These data should be quantified and shown as histograms.
Response: Thank you for your reminding, the "Figure 6" has been substituted by "Figure 4", and the histogram has been added into Figure 4.

The responses to reviewer3 (00698109)
Comment: The authors used C3A cells as hepatocytes in this manuscript. I

wonder the effects of sericin on C3A are same as in hepG2 with the same mechanism and signals?
Response: C3A is a clonal derivative of HepG2, so I think sericin could also promote the cell attachment and proliferation of HepG2, but it still needs to be verified, and the mechanism needs to be explored. In the following study, we are going to verify whether this serum-free medium is suitable for HepG2, and study the effect of sericin on HepG2 and the underlying mechanism.

Comment: The role of sericin in hepatocyte growth and attachment are already known. So if the mechanism is an important finding in the present study, the expression of the gene or signal transducer mentioned by the authors can be confirmed by PCR or western blot or some inhibitors based on the chip results
Response: I can't agree more, that's what we are going to do in the further study. Since the differences on gene expression of some signal transducers have been revealed by gene chip and verified by RT-qPCR in this study, we plan to confirm the *CCR6-Akt-JNK-NF-kB* pathway and *ERK1/2*-MAPK pathway with western blotting and the inhibitors in the following study.

Special thanks to you for your good comments.
Yours sincerely,
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Reference
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2 Tsiaoussis J, Newsome PN, Nelson LJ, Hayes PC, Plevris JN. Which hepatocyte will it be? Hepatocyte choice for bioartificial liver support systems. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2001; **7**(1): 2-10 [PMID: 11150414 DOI: 10.1053/jlts.2001.20845]
3 Choi JM, Oh SJ, Lee SY, Im JH, Oh JM, Ryu CS, Kwak HC, Lee JY, Kang KW, Kim SK. HepG2 cells as an in vitro model for evaluation of cytochrome P450 induction by xenobiotics. *Archives of pharmacal research* 2015; **38**(5): 691-704 [PMID: 25336106 DOI: 10.1007/s12272-014-0502-6]
4 Thompson J, Jones N, Al-Khafaji A, Malik S, Reich D, Munoz S, MacNicholas R, Hassanein T, Teperman L, Stein L, Duarte-Rojo A, Malik R, Adhami T, Asrani S, Shah N, Gaglio P, Duddempudi A, Borg B, Jalan R, Brown R, Patton H, Satoskar R, Rossi S, Parikh A, ElSharkawy A, Mantry P, Sher L, Wolf D, Hart M, Landis C, Wigg A, Habib S, McCaughan G,

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