Peer Review File

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Reviewer Comments

Responses to the Reviewers.

We thank the Reviewers for their comments and suggestions. We did our best to improve our manuscript in accordance with Reviewers' indications.

Point by points answers

Review A

Comment 1: The methods section needs to be expanded what search term were included? Why are certain models/studies included and just as importantly why are some excluded.

Reply1: The methods section has been expanded. See lines 110-117 and Table 1. We now better specified the search terms included and the inclusion and exclusion criteria considered to perform the research.

Comment 2: The structure of the manuscript can be improved – as an example the section on HSC and cytokines can be moved to background. The same applies to subheading –please with clear what that the information in each sub-section matches the heading.

Reply 2: We improved the structure of the manuscript. In particular, paragraphs of HSCs and cytokines have been moved to background (see lines 54-97). We improved the sub-heading divisions, including the right cell types in the corresponding heading and deleting the cell types that were not further investigated in this review (e.g. normal fat-storing cells, cirrhotic fat-storing cells and the biliary stellate cell line). See lines 142-161 and 222-252. We added two new subheading ("Hepatic tumor cell lines as surrogates of hepatocyte activity and metabolism" and "Stem cell-derived HSCs") to better classify the cells used in the different *in vitro* models.

Comment 3: Page 4 – line 122 "and their toxicity is not correlated with animal studies" – what does this mean?

Reply 3: We deleted this sentence. We apologize for this mistake.

Comment 4: Primary cell section – what about immortilised hepatocytes?

Reply 4: We now add a specific subheading entitled "Hepatic tumor cell lines as surrogates of hepatocyte activity and metabolism" where we described the importance of hepatocytes in fibrosis development and reported that primary hepatocytes are difficult to *culture in vitro* for their limited lifespan and, for this reason, hepatic tumor cell lines were used as surrogates of primary hepatocytes. See lines 222-238. We added in Table 2 also this kind of cells.

Comment 5: I am very surprised the authors do not mention kupffer cells anywhere in the cell section?

Reply 5: We mentioned Kupffer cells in the 3D-cultures, where Kupffer cells are usually used in co-culture with hepatocytes, hepatic stellate cells, etc.. to mimic what happens during fibrosis development. See lines 263, 308, 324, 331, 363, 370, 372, 401, 403 and 445.

Comment 6: The section on the 3D models of fibrosis can be expanded and improved.
Reply 6: We did our best to summarize the 3D models of liver fibrosis present in the literature, to our acknowledgment, and based on our research criteria. We classified the 3D models in spheroids and organoids, bio-printed models, bioreactors and liver on a chip, precision cut liver slices and take into consideration the possibility to use decellularized liver matrix as a scaffold. Moreover, we add a recent publication to complete the paragraph of spheroid *in vitro* systems (see lines 329-338).

Comment 7: The conclusion is very basic and not all that informative
Reply 7: We did our best to improve the conclusion section, discussing the advantages and disadvantages of *in vitro* models compared to *in vivo* models.

Review B

Comment 1: Page 2, 46
tested the efficacy of various biological molecules and chemical compounds that may possibly attenuate or revert fibrosis (9,10). However, animal testing is not always predictive of the human physiological response, in terms of efficacy and toxicity (11,12). Advantages and drawbacks of in vivo, animal models, and what advantages in vitro models (mostly human-based) can provide. E.g. Testing new therapeutic modalities such as antibody and RNAi can only be tested in human cell-based in vitro models.
The authors should include a statement in this regards.
Reply 1: We now provide advantages and drawbacks of *in vitro* models compared to *in vivo* models. See lines 502-517.

Comment 2: Page 9, line 262 -292
To date, research studies using micromolding-based spheroids have focused on monitoring the hepatocyte phenotype and function in 3D cultures, while HSC activation has not yet been investigated…..
A recent publication (“A 3D primary human cell-based in vitro model of nonalcoholic steatohepatitis for efficacy testing of clinical drug candidates) focus on the modelling of liver fibrosis in the context of NASH by using a multicell type microtissue system. This reference would complement the paragraph of spheroid based in vitro systems
Reply 2: We add the recent publication (“A 3D primary human cell-based in vitro model of nonalcoholic steatohepatitis for efficacy testing of clinical drug candidates”) to complete the paragraph of spheroid *in vitro* systems. See lines 329-338.

Comment 3: Page 15, line 471

Future optimization of the in vitro 3D models of fibrogenesis would definitely help to identify new anti-fibrogenic compounds and efficient therapy to treat hepatic fibrosis. The authors may, in addition, point out, that in vitro models can significantly reduce the use of animal testing in drug safety and development.

Reply 3: We now point out that the *in vitro* models can significantly reduce the use of animal testing in drug safety and development. See lines 512-517.