Three-dimensional bioprinted hepatorganoids in liver failure

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We read with interest the recent study by Yang et al, who reported the construction of three-dimensional bioprinted hepatorganoids (3DP-HOs) using HepaRG cells and bioinks.1 We congratulate the authors for this pioneering study that demonstrates the fabrication and in vivo application of functional 3DP-HOs in mice models of liver failure. The study would pave the way for the use of three-dimensional (3D) bioprinted primary hepatocytes and/or hepatic progenitor cells as possible regenerative therapies in liver diseases. However, there are some caveats in the study, which need to be highlighted.

First, the study showed that after 7 days of differentiation in vitro, HepaRG cells in 3DP-HOs expressed hepatic marker genes and exhibited albumin secretion (figure 2). However, it is to be noted that most of the HepaRG cells embedded in alginate-gelatin bioink in 3DP-HOs seem to be dispersed and single with a very few aggregates/clusters of cells (figure 1D). In such a case, the gene expression and functional studies performed in the cells can be misleading and erroneous.2 The authors should highlight this concern.

Second, the authors reported that the hepatic gene expression and functions of cells in 3DP-HOs were comparable to those observed in primary human hepatocytes (PHHs) in two-dimensional (2D) cultures. It has been well documented that PHHs de-differentiate within 1–2 days and subsequently show declined hepatocyte-specific gene expression and functions in conventional 2D cultures (3). In fact, most of the strategies including 3D cultures and cocultures of primary hepatocytes with non-parenchymal cells are undertaken to enhance the functions of PHHs in vitro. The authors themselves discuss in the manuscript that HepaRG cell lines possess metabolic and morphological properties that are comparable to PHHs and thus comparing the gene expression and functions of cells in 3DP-HOs with PHHs does not seem correct.3 Given the 3D structure of
3DP-HOs, to underscore the hepatic features of 3DP-HOs, a more robust model would be to compare their hepatic gene expression with human liver tissues instead of comparing them with PHH monolayer cultures.\(^4\)

Next, the study demonstrated that after transplantation into abdominal cavity of Fah\(^{−/−}\)Rag2\(^{−/−}\)mouse model of liver injury, 3DP-HOs displayed decreased markers of liver injury and also had enhanced survival (figure 3A,C). The authors have used three groups of mice for comparison here, wild type, sham and 3DP-HOs. Here, another group of mice transplanted only with dispersed HepaRG cells would have added another level of validation to ascertain that the in vivo protective effects obtained in the mice were due to the use of oxygen-permeable hydrogel encapsulated 3D bioprinted HepaRG cells and not due to HepaRG cells alone.

Also, although the authors illustrated a significant improvement in survival and liver functions with resolution of liver injury in the mice transplanted with only a few hepatocytes in 3DP-HOs, they have not made it clear for how long the transplanted 3DP-HOs survived in vivo. This information would shed light on whether the improvement in liver functions in the injured mice treated with 3DP-HOs was due to enhanced survival/viability or due to the increased functions of hepatic cells in the 3D bioprinted organoids.

A clarification of these issues by the authors would provide further insights and a better understanding for constructing and translating 3D bioprinted organoids into the clinical arena.

References


Footnotes

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