Functional assessment of 3D bioprinted liver tissues from human pluripotent stem cells

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INTRODUCTION

Cell engineering, facilitated by 3D printing, enables the creation of complex morphologies emulating the biological milieu, including the extracellular matrix (ECM). Currently, this field holds significant promise for drug screening and therapeutic applications. However, previous attempts at 3D bioprinted liver tissues mainly relied on immortalized hepatic cells, resulting in a lack of manifestation of the native liver functions. In this study, we successfully generated hepatic progenitor cells (hepatoblast: HB) and mature functional hepatocytes from human pluripotent stem cells to evaluate their functionalities after 3D bioprinting.

MATERIALS AND METHODS

hPSC-derived hepatoblasts (hPSC-HBs) were differentiated following our published protocols [1], [2]. Subsequently, hPSC-HBs were further induced to promote functional mature hepatocytes, characterized by AFP-negative and ALB-positive cells. Moreover, we achieved hepatic functional heterogeneity representing zonated difference by manipulating the Wnt signal pathway, resulting in the differentiation of Zone 1 (peri portal hepatocytes) like cells and Zone 3 (peri central hepatocytes) like cells. These hepatic cells formed the organoids for four days before being encapsulated in the hydrogel. Comparisons were made between 3D hepatic organoids embedded using conventional manual encapsulation in matrigel and those 3D bioprinted liver organoids printed by CellInk BioX. Cell viability was examined by Live/Dead staining (Invitorgen), and albumin secretion was evaluated by ELISA. RNA was isolated, and gene expression was assessed by qPCR. Bioprinted tissues were fixed, and liver-specific markers, including ALB, AFP, CK8/18, and ASGPR were stained by immunohistochemistry.

RESULTS AND DISCUSSION

Different numbers of hPSC-HB organoids were encapsulated in 10ul matrigel manual droplets, and the albumin secretion levels were measured by ELISA at 10 days. The albumin secretion increased with the number of organoids, 15.62 ± 3.02, 22.66 ± 4.50, 27.46 ± 6.49, 31.14 ± 9.53 ug/ml (mean ± SD with the cell densities of $0.25X10^5$, $0.5X10^5$, $1.0X10^5$, $2.0X10^5$ respectively. Encouragingly, comparable albumin secretion was observed when cells were encapsulated by 3D printing. The 3D bioprinted Zone1 and Zone3 mature hepatocytes demonstrated high viability of organoids post 3D bioprinting with the proper level of human albumin secretion even when many organoids were printed. Currently, we are assessing *in vitro* hepatic functions to evaluate drug metabolism functionality in 3D bio-printed hPSC- derived liver tissue with different zone-like hepatocytes.

CONCLUSIONS

3D-printed hPSC-derived liver organoids exhibited viable cells and maintained liver functions. These complexed liver tissue comprising hPSC-derived hepatocytes represent a valuable *in vitro* model for developing future therapeutic interventions.

ETHICAL STATEMENTS

hPSC-derived cells were obtained commercially from WiCell (Madison WI, USA).

REFERENCES

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