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DEVELOPMENT OF IMMUNE-EVASIVE META-SOFT ORGAN MODULES THROUGH ADVANCED CELL ENGINEERING AND ASSEMBLY TECHNOLOGY

Hee Ju KIM [1], Jiyoon Byeon BYEON [1], **Chae Eun LEE** [1], Songwan JIN [2], Gyeong Woon KONG [2], Youngju SON [3], Kangin LEE [3], Jae Young LEE [3], Jinah JANG [4], Dayoon KANG [4], Daekeun KIM [4], Donghwan KIM [4], Ji Hyun SHIN [1], Dongho CHOI [1]

[1] *Biology, Esearch Institute of Regenerative Medicine And Stem Cells, Hanyang University,, Republic of Korea,* [2] *Biology, T&R Biofab Co. LTD,, Republic of Korea,* [3] *Biology, Therapeutics R&D, ToolGen Inc, Republic of Korea,* [4] *3D Organ Printing, Pohang University of Science And Technology (POSTECH), Republic of Korea*

Background : Immune rejection and donor shortages pose challenges in liver transplantation. This study addresses these barriers by engineering immune-evasive Meta-iPSC via HLA knockout and immune checkpoint molecule knock-in to improve transplantation outcomes and advance regenerative medicine.

Methods : Using CRISPR-Cas9 genome-editing technology, TnRhipsc-4F cells were modified via HLA gene knockout and immune checkpoint molecule knock-in to generate ALC3-3 cells capable of inducing immune tolerance. These cells were differentiated into Meta-iPSC-derived hepatocytes over a 20-day period. Hepatocyte-specific gene expression was validated through RT-PCR, while immunocytochemistry confirmed the protein expression of hepatocyte markers. Albumin secretion was quantified using flow cytometry (FACS). The differentiated hepatocytes were then assembled into three-dimensional organ modules with vascular structures and transplanted into rabbit livers to evaluate in vivo biocompatibility.

Results : Pluripotency markers in the undifferentiated Meta-iPSC ALC3-3 state and hepatocyte-specific markers in Meta-iPSC-derived hepatocytes were verified via RT-PCR and immunocytochemistry. FACS analysis showed albumin and MRP2 expression in over 80% of Meta-iPSC-derived hepatocytes. Post-transplantation analyses, including DAB staining, confirmed the expression of human-specific markers such as hGAPDH and hALB in rabbit liver tissues, demonstrating successful engraftment and functional integration of the immune-evasive cells.

Conclusions : The successful differentiation and characterization of Meta-iPSC-derived hepatocytes, along with their assembly into bioengineered organ modules, were validated through extensive in vitro and in vivo analyses. Transplantation into a rabbit model confirmed their biocompatibility and functionality. This approach marks a transformative step in addressing key barriers in organ transplantation, such as immune rejection, and highlights the potential for future advancements in regenerative medicine.

Corresponding Author : **Ji Hyun SHIN** (crane87@hanyang.ac.kr)