A novel strategy for treating acute liver failure: encapsulated proliferating human hepatocyte organoids

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Acute liver failure (ALF) is a short-term severe damage to hepatocytes caused by hepatotropic viruses, drug, etc. and accompanied by complications such as multi-organ failure and hepatic encephalopathy.[1] The global incidence of ALF is estimated at over 1 million cases annually.[2] Liver transplantation is currently the sole curative treatment for ALF, but due to the limitations of donor organ shortage, complications and long-term immunosuppression, hepatocytes transplantation has been proposed as an alternative strategy.[3] However, the shortage of human primary liver cells (PHHs) and the difficulty in amplification *in vitro* hinder their clinical application. Stem cells can provide an abundant source of cells for the treatment of ALF, but the hepatocytes derived from them have limitations in expansion, insufficient liver function and safety problems. Three-dimensional organoids constructed from stem cells or organ-specific cells play an important role in liver regeneration research.[4] PHHs can be converted into a bi-phenotypic state with expandable property *in vitro* and express markers of mature hepatocytes and hepatocyte progenitors,[5] which can be re-differentiated into mature hepatocytes with significant enhancements in liver function when cultured further into organoids.[6] Nevertheless, the immune rejection of organoids generated by proliferating human hepatocytes (ProliHHs) after transplantation, as well as safety issues, remain unresolved and require further research.

In *Cell Stem Cell*, Hui et al.[7] from the CAS Center for Excellence in Molecular Cell Science demonstrated that the large-scale expanded alginate-encapsulated human liver organoids (eLO) with quality control could alleviate liver injury and facilitate liver regeneration by maintaining the stability of the gut-liver axis, protecting the intestinal barrier, reducing endotoxins originating from the intestines. The team also verified the genomic stability of ProliHH cells and the *in vivo* safety of eLO transplantation (**Figure 1**). The dedifferentiation and large-scale amplification techniques of PHHs are notable highlights of this study. Hui et al.[5] had previously developed a chemically defined medium, which could dedifferentiate PHHs *in vitro* without genetic manipulation and could generate ProliHHs with the characteristics of both hepatocytes and progenitor cells. The ProliHHs could be expanded by at least 10,000-fold during the culture *in vitro*. After transplanted into immune-deficient *Fah*$^{-/-}$ mice, 5×10^5 ProliHHs could regenerate approximately 60% of the damaged liver, demonstrating strong therapeutic potential. In this study, after further differentiation and maturation in three-dimensional organoid system, liver organoids derived from ProliHHs expressed liver-related genes and had mature liver cell functions like cytochrome P450 metabolism, urea synthesis, and serum albumin production. Proliferating human hepatocyte organoids effectively addressed the scarcity and batch effect of PHHs in transplantation therapies for diseases. However, the transplantation of organoids alone faces the challenge of host immune rejection. Hydrogels utilized as carriers for encapsulating grafts can effectively solve this issue.

Alginate, a type of hydrogel suitable for cell microencapsulation technology, exhibits excellent biocompatibility, physical stability and ease of gelation.[8, 9] Some preclinical and clinical studies showed that alginate-encapsulated PHHs represented a promising transplantation strategy for liver failure therapy.[10, 11] In this study, the application of ultra-pure alginate encapsulation for organoids derived from ProliHHs creates an immune-protective and semi-permeable barrier,[12] thereby preserving the cellular activity of the internal liver organoids. After the implantation of eLO into the peritoneal cavity of

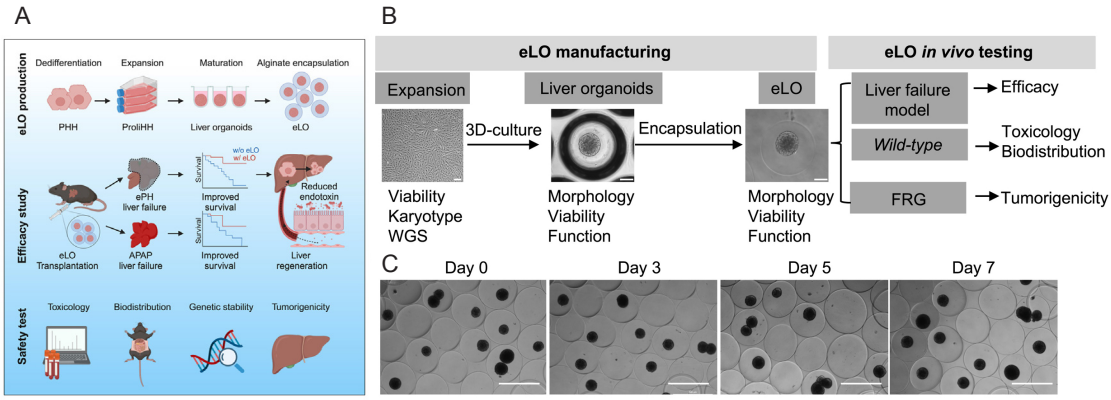

Figure 1. (A) Human primary hepatocytes were dedifferentiated, expanded, and matured to form liver organoids, which were subsequently encapsulated in eLO with alginate. eLO transplantation into ePH and APAP induced ALF mice improved survival, reduced endotoxin levels, and promoted liver regeneration; safety test: including toxicity and biodistribution of eLO, as well as the genetic stability and tumourigenicity of ProliHHs. Reprinted from Hui et al. (B) Schematic diagram of the experimental design depicting the generation and application of eLO. Scale bars: 100 μm. (C) Representative images of eLO during 7-day culture. Scale bars: 100 μm. Reprinted from Hui et al.[7] APAP: acetaminophen; eLO: encapsulated proliferating hepatocytes-derived liver organoids; ePH: extended 80% partial hepatectomy; FRG: *Fah*$^{-/-}$*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice; PHHs: primary human hepatocytes; ProliHHs: proliferating human hepatocytes; WGS: Whole-genome sequencing.

ALF mice induced by ePH (extended 80% partial hepatectomy), it was observed that most of eLO presented a smooth surface without fibroblast deposition by day 7, indicating the high biocompatibility of alginate despite some cases of fibroblast adhesion. Furthermore, the survival rate of encapsulated liver organoids exceeded 80% on day 7. The team also analyzed the acute toxicity, and biodistribution of eLO and the genetic stability and tumourigenicity of ProliHHs to confirm the safety of clinical application[7].

In this study, PHHs were dedifferentiated into a bi-phenotypic ProliHHs without genetic manipulation. This not only tackled the problems of insufficient primary hepatocyte sources and inconsistent batch quality but also constituted a significant advantage of eLO in terms of cell therapy safety. The hydrogel carrier technology, specifically ultra-pure alginate encapsulation, can offer immune protection and a semi-permeable barrier for the transplanted organoids. Simultaneously, this article also pointed out certain limitations. The source of donor cells was limited to one adult donor and several pediatric donors, with no explicit criteria established for donor selection in clinical applications. Although supplementing 5% of the total liver mass could rescue mice from ePH and acetaminophen-induced ALF, further validation was still required to determine the optimal cell dosage for treatment in subsequent clinical studies. In Addition, we believe that there are still some aspects that need further discussion. It will be better that the quality standards of ProliHHs and eLO is presented for future clinical applications, and the best method of transplantation need to be further confirmed to develop longer-term effectiveness *in vivo*. Furthermore, the establishment of a ProliHHs bank from diverse donors should be contemplated for future large-scale applications, along with evaluating the necessity of matching before transplantation. Moreover, regarding the safety studies such as toxicity analysis of ProliHHs and eLO, it is advisable to illustrate that these have been validated by Good Laboratory Practice. Finally, further in-depth research on the mechanisms of eLO in the treatment of liver failure is warranted.

Author contributions

XM and AL conceived and wrote the draft; OP edited and reviewed the manuscript; YS conceived and modified the manuscript. All authors read and approved the final manuscript.

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Conflicts of interests statement

The authors declare no conflicts of interest.

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