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**Clinical translation of bioartificial liver support systems with human pluripotent stem cell-derived hepatic cells**

Sakiyama R *et al*. Bioartificial liver with human iPS

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**Abstract**

There is currently a pressing need for alternative therapies to liver transplantation. The number of patients waiting for a liver transplant is substantially higher than the number of transplantable donor livers, resulting in a long waiting time and a high waiting list mortality. An extracorporeal liver support system is one possible approach to overcome this problem. However, the ideal cell source for developing bioartificial liver (BAL) support systems has yet to be determined. Recent advancements in stem cell technology allow researchers to generate highly functional hepatocyte-like cells from human pluripotent stem cells (hPSCs). In this mini-review, we summarize previous clinical trials with different BAL systems, and discuss advantages of and potential obstacles to utilizing hPSC-derived hepatic cells in clinical-scale BAL systems.

**Key words**:Artificial liver; clinical trial; hepatocytes; pluripotent stem cells; bioreactors

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**Core tip:** The current lack of transplantable donor livers in the world has led to the development of extracorporeal liver support systems as one possible approach to overcome this problem. Bioartificial liver (BAL) support systems require a cell source to replicate human liver function, yet the ideal cell source for this purpose has yet to be determined. Highly-functional hepatocyte-like cells have recently been generated from human pluripotent stem cells, which show promise as a potential cell source in BAL support systems for the treatment of liver failure in the future.

Sakiyama R, Blau BJ, Miki T. Clinical translation of bioartificial liver support systems with human pluripotent stem cell-derived hepatic cells. *World J Gastroenterol* 2017; In press

**InTRODUCTION**

***Needs for bioartificial liver systems in clinical practice***

Liver disease is one of the most prevalent medical conditions in the world today, affecting hundreds of millions of people worldwide[[1](#_ENREF_1)-3]. Many of these diseases, such as end-stage liver diseases and some inherited liver diseases, can only be treated successfully with a liver transplant[[4](#_ENREF_4)]. Although 11606 patients were added to the liver transplant waiting list in the year 2015, only 7127 patients received a liver transplant in that same year[[4](#_ENREF_4)]. This discrepancy demonstrates the profound shortage of transplantable donor livers. This shortage of livers resulted in a high waiting list mortality, with 1423 patients dying in 2015 while waiting for a transplant[[4](#_ENREF_4)]. Therefore, it is imperative that new therapies are developed to provide an alternative to liver transplantation.

Extracorporeal liver support systems were developed with the aim of stabilizing a patient long enough for his or her own liver to regenerate or for physicians to procure a transplantable liver. Early support systems functioned to supplement liver function by removing toxins from the blood through non-biological hemofiltration[[5](#_ENREF_5)]. These non-biological type extracorporeal liver support systems have been clinically established and are widely used in countries where liver transplantation is limited[[5](#_ENREF_5)]. However, it became apparent that non-biological hemofiltration devices were incapable of adequately replicating liver function [[5](#_ENREF_5)]. In order to overcome the limitations of non-biological devices, live cells that possess liver function were incorporated into the development of bioartificial liver support (BAL) systems[[5](#_ENREF_5)]. There are several types of BAL systems that have been proposed which differ in their cell housing mechanism, including hollow fiber-based[[6-10](#_ENREF_6)], multilayer membrane-based[[11](#_ENREF_11)], sponge/scaffold-based[[12](#_ENREF_12)-[14](#_ENREF_13)], and floating/encapsulated-based systems[[15](#_ENREF_15)] (Table 1). Although most of these housing mechanisms have successfully cultured cells on the small experimental scale, hollow fiber-based BAL systems are widely used in clinical trials.

**Sources of hepatocytes for BAL systems**

Several types of cells may be selected for use in a BAL system. These include primary hepatocytes isolated from human livers, human hepatoblastoma cell lines, and primary animal hepatocytes[[16](#_ENREF_16)]. Human primary hepatocytes are ideal for the BAL system[[16](#_ENREF_16)]. However, the low availability and inconsistent quality of primary human hepatocytes prevent their use in clinics[[16](#_ENREF_16)]. Although human hepatic cancer cell lines and animal liver cells are readily available, they are less metabolically active than primary human hepatocytes[[17](#_ENREF_17)]. In addition, the risk of zoonoses precludes the use of animal cells. For example, it has been shown that porcine endogenous retroviruses are capable of infecting human cells *in vitro*[[18](#_ENREF_18)].

Recent advancements in stem cell research have demonstrated that hepatocyte-like cells can be derived from human pluripotent stem cells (hPSCs)[[19](#_ENREF_19)]. hPSCs can be generated from a patient’s own cells by introducing several transcription factors[[20](#_ENREF_20)]. They are capable of differentiating into cells from all three germ layers, including neural cells[[21](#_ENREF_21)-23], osteogenic cells[[24](#_ENREF_24)], cardiac cells[[25](#_ENREF_25)] , adipogenic cells[[26](#_ENREF_26)], pancreatic cells[[27](#_ENREF_27),28], vascular cells[[29](#_ENREF_29)], hematopoietic cells[[30](#_ENREF_30)], endothelial cells[[30](#_ENREF_30)], and hepatocytes[[31](#_ENREF_31),32]. hPSC-derived hepatic cells have been shown to express hepatocyte marker genes and proteins[[33](#_ENREF_33)]. They also demonstrate hepatic functions including albumin secretion, urea synthesis, cytochrome P450 enzyme induction[[31](#_ENREF_31)], and glycogen storage[[34](#_ENREF_34)].

hPSC-derived hepatic cells possess minimal risk when used in a bioartificial liver system, but are unsuitable for other applications due to their risk of tumorigenicity. The genetic instability of hPSCs results in an underlying uncertainty of transplanting large quantities of hPSC-derived hepatic cells directly into a patient[[35](#_ENREF_35)]. On the other hand, the risk of tumorigenicity is minimized in a bioartificial liver system, as the hPSC-derived hepatic cells would be isolated from the patient’s blood stream by multiple layers of filter membranes (Figure 1). Therefore, while hPSC-derived hepatic cells may not be ideal for cell transplantation, they are viable candidates for a bioartificial liver system.

**Successes and challenges of developing clinical BAL systems**

Several BALs have been evaluated in clinical trials, as previously explored in van de Kerkhove *et al*[13,14] (Table 2)[[6-10](#_ENREF_6),[13](#_ENREF_13),[14](#_ENREF_14)]. The Extracorporeal Liver Assist Device (ELAD) utilizes the human hepatoblastoma cell line HepG2/C3A (100 g) in hollow fiber-based dialysis cartridges. A phase III trial treated 96 patients with alcohol-induced liver decompensation. In subjects age < 50 years, creatinine < 1.3 mg/dL, bilirubin ≥ 16 and international normalized ratio (INR) ≤ 2.5, the 91-d survival rates were 93.9% for ELAD-treated subjects and 68.4% for control subjects (*P* = 0.006)[[10](#_ENREF_10)]. A second BAL design, the Modular Extracorporeal Liver Support (MELS) system, consists of interwoven hollow fiber membranes, creating a three-dimensional framework utilizing primary human hepatocytes. In one trial, eight patients (two with acute liver failure, four with acute-on-chronic liver failure, and two with primary nonfunction) were successfully bridged to liver transplantation[[8](#_ENREF_8)]. Several other trials have yielded similar results regarding degree of effectiveness.

Despite the effectiveness of BAL systems in clinical trials, their translation from the laboratory bench to the patient’s bedside has been hindered by three obstacles. Firstly, it is necessary to prepare a sufficient quantity of hPSC-derived hepatic cells for clinical applications. It has been widely suggested that approximately 30% of the total liver volume is required for survival. Considering that the average mass of a human liver is 1.5 kg, and that 100 million hepatocytes are contained in 1 g of liver tissue, a minimum of 45 billion hPSC-derived hepatic cells would be required to produce a clinical-scale bioartificial liver device[[36](#_ENREF_36)] (Figure 2). Secondly, the operation cost of a bioartificial liver device is currently too expensive for widespread clinical use. The process of culturing 45 billion hPSCs and inducing hepatic differentiation consumes large quantities of culture medium and supplements including recombinant growth factors[[37](#_ENREF_37)]. As the length of treatment increases, the cost of operating a BAL device accumulates significantly. Thirdly, it has not been well investigated whether hPSC-derived hepatic cells maintain their liver functions over a long period of time in BAL devices. The loss of cell viability and functionality throughout the course of treatment may be problematic[[38](#_ENREF_38)].

The most critical factor for large-scale cell culture is oxygen and nutrient supply. The oxygen and nutrients must be uniformly supplied to a large number of cells. It is well known that the anchorage-dependent hepatocytes easily form aggregates, and if the diameter of the aggregates exceeds 100µm at atmospheric concentrations, central necrosis occurs resulting from lack of oxygen and nutrition[[39](#_ENREF_39)]. This fact indicates that the organization of the cell culture space in the large-scale BAL system must allow for sufficient oxygen and nutrient penetration of the cell aggregates. A sophisticated controlling system and well-engineered bioreactor will be required to monitor oxygen and nutrient supply. In addition, since hPSCs are sensitive to environmental factors, the shear stress from the culture medium must be minimized[[40](#_ENREF_40)]. Ideally, the bioreactor should mimic the structure within the liver, which provides appropriate pressure and shear stress similar to the Space of Disse.

**Conclusion**

BAL systems have demonstrated a potential to treat patients with liver failure by providing temporary support for them to recover their own hepatocytes or to bridge them to liver transplantation. Early BAL systems have encountered significant limitations due to the low functionality and availability of cells for this application. With emerging stem cell technology, hepatocyte-like cells can be differentiated from hPSCs. Due to their functional similarity to primary human hepatocytes and minimal risk of use, these hPSC-derived hepatic cells will be the ideal cell source to develop clinical-grade bioartificial devices. Further clinical translational studies will be required to overcome the obstacles to developing large-scale bioartificial liver devices with hPSC-derived hepatic cells. If successful, these readily available and highly functional extracorporeal liver support systems will be a feasible alternative for the treatment of liver failure in the near future.

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**Figure 1 Bioartificial liver system with human pluripotent stem cells-derived hepatic cells using double filtration plasmapheresis.** In a bioartificial liver (BAL) system, patient plasma is first separated from whole blood by double filtration plasmapheresis (DFPP). Plasma then perfuses a bioartificial device using hydrophilic hollow fibers. The human pluripotent stem cells (hPSCs)-derived hepatic cells are inoculated at the outside of the hollow fibers. The detoxified patient plasma is filtered once more before returning to the patient’s blood stream. The hollow fiber membranes and safety filter provide two layers of separation between the patient’s blood stream and the hPSC-derived hepatic cells.

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**Figure 2 A strategy and cell number estimate of human pluripotent stem cells-derived hepatic cells in the mass production of bioartificial liver devices.** Undifferentiated human pluripotent stem cells (hPSCs) can be expanded in a 15 L suspension culture system up to a maximum of 15 billion cells[[37](#_ENREF_37)]. Three of these suspension culture flasks will be required to prepare 45 billion cells for a clinical-scale bioartificial liver (BAL) device. After inducing hepatic differentiation, the hPSC-derived hepatic cells will be cultured at high density in bioreactors to generate a BAL device.

**Table 1 Artificial liver device designs**

|  |  |  |
| --- | --- | --- |
| **Type** | **Pros** | **Cons** |
| Hollow fiber (tubular) | Simple structure, divertible from dialyzer, minimal shear stress, immunoisolation | Uneven gas-liquid mass transfer, no intrinsic oxygen supply |
| Hollow fiber (interwoven) | Ease of scale-up, efficient and uniform mass transport, minimal shear stress, immunoisolation, good oxygen and nutrient supply | Complex structure |
| Multilayer membrane | Uniform cell distribution and microenvironment | Limitations to scale-up, cells exposed to direct shear stress, low surface area-to-volume ratio, no intrinsic oxygen supply |
| Sponge/Scaffolds | Ease of scale-up, minimal barrier to nutrient/metabolite transport | Non-uniform cell distribution, cells exposed to shear stress, no intrinsic oxygen supply |
| Floating/Encapsulated | Ease of scale-up, uniform microenvironment | Poor cell stability, barrier to nutrient/metabolite transport due to encapsulation, degradation of microcapsules over time, no intrinsic oxygen supply |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Bioreactor device** | **Ref.** | **Cells** | **Mass (g)1** | **Bioreactor design** | **Scaffold** | **Fluid** | **Separation** | **Treatment time (h)** | **Phase** | **Indication (*n*)** | **Effect** |
| HepatAssist | Demetriou *et al*[9] | Cryopreserved porcine hepatocytes | 50-70 | Hollow fiber | Microcarrier + external inoculation | Plasma | 3000 kDa cut-off | 6 | III | ALF (147), PNF (24) | HepatAssist survival of 71.0% *vs* control survival of 62.0% *p* = 0.28, (N.S.) |
| Vitagen ELAD | Reich *et al*[10] | HepG2/C3A | 200-400 | Hollow fiber | External inoculation | Plasma | 70 kDa cut-off | Up to 168 | III | AILD (96) | ELAD survival of 80.4% *vs* control survival of 65.2%  *p* = 0.068, (N.S.) |
| LSS | Mundt *et al*[7] | Primary porcine hepatocytes | up to 500 | Hollow fiber | External inoculation | Plasma | 300 kDa cut-off | 7-46 | I/II | ALF (8) | Bridged to OLT 8 |
| MELS | Sauer *et al*[8] | Primary human hepatocytes | up to 600 | Hollow fiber | External inoculation | Plasma | 400 kDa cut-off | 7-74 | I | ALF (2), PNF (2), AOC(4) | Bridged to OLT 6, Survival without OLT 1, Died without OLT 1 |
| Excorp Medical BLSS | Mazariegos  *et al*[6] | Primary porcine hepatocytes | 70-120 | Hollow fiber | Collagen + external inoculation | Whole blood | 100 kDa cut-off | 12 | I | ALF (2), AOC (2) | Bridged to OLT 1, Died without OLT 3 |
| AMC-BAL | van de Kerkhove  *et al*[13,14] | Primary porcine hepatocytes | 100 | Nonwoven | Spiral membrane + polyester matrix | Plasma | None | 24 | I | ALF (12) | Bridged to OLT 11, Survival without OLT 1 |

**Table 2 bioartificial liver devices used in clinical trials**

1100 million cells/gram of liver. AILD: Alcohol-Induced Liver Decompensation; AOC: acute-on-chronic liver failure; ALF: acute liver failure; PNF: primary graft nonfunction; OLT: orthotopic liver transplantation.