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SUPPLIVER: Bioartificial supply for liver failure

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Abstract

1) Objectives

The SUPPLIVER project aims at developing a liver supply system based on cell encapsulation in alginate beads.

2) Material and methods

After hepatic cells and alginate were mixed, beads were produced by an extrusion method that made the droplets fall in a gelation bath. A bioreactor was designed to host the beads under a fluidized bed perfusion regimen. The biological components were inserted in a complete and safe extracorporeal circuit based on a therapeutic plasmapheresis device. In vitro trials were performed to assess biological functions and both ex vivo and preliminary in vivo studies focused on the system's safety.

3) Results

The encapsulation and fluidization processes were validated, showing the maintenance of hepatic functions once spheroids of hepatocytes were formed before encapsulation. The whole extracorporeal circuit was built, including all the monitoring processes for priming and treatment. The first preclinical trials were successful on a sheep model.

4) Conclusion

The multidisciplinary consortium succeeded in demonstrating the feasibility of the proposed integrated approach, from cell collection to extracorporeal circuit functions. It led to a promising combined advanced therapies medicinal product, that still needs to be challenged in a large model of hepatic failure.

1. Introduction

The liver is the largest and most metabolically complex organ in the body. The World Health Organization estimates that over 650 million people worldwide are affected by some forms of liver disease. On a worldwide base, 1–2 million deaths are accounted to liver related diseases annually. In the US alone, there are around 500,000 critical episodes of liver problems requiring hospitalization with 80,000 deaths annually.

Liver failure results from the liver's inability to perform its normal functions. It is a severe clinical syndrome in which the liver's metabolic functions – detoxification, biotransformation, excretion and synthesis – are severely impaired leading to the accumulation of lethal toxins in the patient and the onset of life-threatening complications and manifestations.

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Liver failure is associated with a rise in numerous endogenous substances such as bilirubin, ammonia, glutamine, lactate, aromatic amino acids, free fatty acids, phenol, mercaptans, benzodiazepines and proinflammatory cytokines [1]. The loss of liver function appears to result from an overload of hepatotoxic substances that progressively saturate available detoxification pathways, leading to the accumulation of other toxins and the production of cytokines. Moreover, the accumulation of toxins may further impair the patient's liver as a result of hepatocellular apoptosis and necrosis. The most frequent complications of liver failure are hepatic encephalopathy (HE), coagulopathy, jaundice, cholestasis, pruritus, ascites, immune disorders, sepsis and kidney failure.

In acute liver failure (ALF), liver function is normal 2 to 8 weeks before the onset of the disease. In acute-on-chronic liver failure (A-on-C-LF), liver function decreases abruptly in a patient already suffering from chronic liver insufficiency [2]. The most frequent causes of ALF are intoxications, especially acetaminophen intoxication, viral infections and hepatic ischemia. Sometimes the cause remains unknown [3]. A-on-C-LF generally occurs in patients with cirrhosis, following infectious disorders, toxin exposure or gastrointestinal bleeding. Both are associated with high morbidity and mortality without transplantation.

If liver transplantation remains the only efficient treatment for patients suffering from acute or fulminant organ failure, its cost, the scarcity of organs and the surgical and post-surgical complications resulting from chronic immunosuppression strongly limit the number of patients that may benefit from this procedure. The shortage in donors has resulted in a high death rate among the potential patients waiting for a graft. In France, for about 1100 transplantations per year, there are more than 1500 new inscriptions, and 6–7% die on the waiting list [4]. In 2013, only 1562 patients were transplanted among the 2041 individuals on the EU waiting list for liver transplant [5]. For patients with fulminant hepatic failure, a severe liver disease with 60–90% mortality, only 10% received a transplant. Liver transplantation has also a relatively high mortality of 30–40% at 5–8 years with 65% of the deaths occurring in the first 6 months. In addition, patients who have undergone transplantation have to use lifelong immunosuppressive therapy.

For the past 20 years, this expanding gap between the number of patients on waiting list and the number of transplants has highlighted the requirement for a temporary liver support. Such an artificial organ could be employed either as a bridge to transplantation or regeneration.

Extracorporeal liver support devices have therefore been developed in the last few decades in order to either bridge patients to liver transplantation or allow the native liver to recover from injury. They may also be valuable when primary non-function occurs after liver transplantation or when a large hepatic resection leaves too little liver in reserve.

There are two types of extracorporeal liver assist devices: artificial and bioartificial livers [6].

On the one hand, artificial liver (AL) devices use non-living components to cleanse the blood or plasma of its toxins. Removal is based on physical/chemical gradients and adsorption. At the moment, the two major actors in the field of hemodialysis (i.e. artificial kidney) produce and commercialize their own system, adapted from former monitors developed for plasma or blood filtration (Prometheus® from Fresenius Medical Care, and MARS® from Gambro). In 2010, around 3000 MARS® treatments have been performed in Europe (550 in France). Such devices are helpful in some cases but are not relevant enough to replace all the liver functions.

On the other hand, extracorporeal bioartificial livers (EBAL) contain a cell-housing bioreactor, the role of which is to replace the primary and most important liver functions (oxidative detoxification, biotransformation, excretion and synthesis). In such a bioreactor, cells need to be encapsulated in a specific scaffold to benefit from an efficient anchorage and to be protected against the patient's immunological factors.

Very recently, implantation of hepatocytes was also proposed as a potential treatment of acute liver failure. The cell encapsulation, already investigated in EBAL, could also be a valuable approach in the case of implantable bioartificial liver (IBAL).

As an alternative to the above mentioned BAL, SUPPLIVER proposes to encapsulate human liver cells in a spherical porous structure (alginate beads modified or not), instead of using more traditional techniques based on hollow fibers. Through this approach, it should become possible to prepare a sufficient number of human hepatocytes encapsulated, ready to use, and cryopreserved for storage on the long-term. The encapsulated cells can then either be put in contact with the patient's plasma in a fluidized bed bioreactor, which optimizes the transfer of material and functions of the reconstructed tissue (extracorporeal), or implanted (Fig. 1).

This essentially multi-disciplinary project gathers, around the coordinator (UMR CNRS 7338 Biomechanics and Bioengineering at University of Technology of Compiègne (UTC)) and the R&D center of the manufacturer Gambro (Meyzieu), clinicians and researchers from the Hepatobiliary Center at Villejuif (INSERM U785-Hopital Paul Brousse) and from the Institute for Research in Biotherapy of Montpellier (INSERM U1040) as well as SME Kaly-Cell (cell provider) and the Biobanque de Picardie, a biological resources center located in Amiens Hospital.

2. Material and methods

2.1. Cells

Primary human hepatocytes (PHH), fresh or cryopreserved, were planned to be used in the whole set-up. Nevertheless, to validate a number of processes, other cell types were also employed during the project: C3A [HepG2/C3A, derivative Hep G2] human cell line provided by the American Type Culture Collection (ATCC, reference CRL 10-741), and freshly isolated hepatocytes from 5-week-old male Sprague–Dawley rats obtained from Janvier (Le Genest-Saint-Isle, France). PHH were obtained from Kaly-Cell, the Biobanque de Picardie (BB-0033-00017) and from Montpellier hospital, following the