Relative cost of hepatocytes
- WORKPACKAGE 8
PROGENITOR CELLS FOR BI O-
ARTIFICIAL LIVER
UNEW, Charité, SCS
WP1 Clinical, application scenarios

WP2 System design and medical device regulatory requirements

WP3 Sensor development

WP4 Microfluidics, packaging and integration

WP5 Development and monitoring of cell-based Liver Support Unit

WP6 Instrumentation platforms

WP7 Communications, patient management and decision support

WP8 Progenitor cells for bio-artificial liver

WP9 Dissemination, training and exploitation plans

WP10 Consortium Management

Partners: UNEW, Charité, SCS

Period 1:
Develop experimental extracorporeal liver system
Purification and characterisation of human pancreatic hepatocyte progenitors
Workpackage progress - Introduction

- Primary human hepatocytes
- iPS / ESC-derived hepatocytes
  - difficult to genetically manipulate
  - 10 years
  - EXPENSE
  - B-13 - serendipitous rat hepatocyte progenitor
In the absence of sufficient human hepatocytes from donor livers, the main approach examined for generating human hepatocytes is through differentiation of human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Despite significant progress over the last 12 years, it has not been possible to generate hepatocytes with function quantitatively similar to adult human hepatocytes. It is widely reported that hepatocytes remain in a foetal state and cannot progress further unless transplanted in vivo. Since normal hepatocytes de-differentiate into a foetal state in vitro (even when present within culture tissue slices), it may be not be surprising that this barrier exists in stem cell-derived hepatocyte culture [1]. In addition, a major hurdle to the use of ESC/iPS-derived hepatocyte is their high cost of generation. When a high level of functionality is required (e.g. if the proposed use is in an extracorporeal device), then this significantly impacts on cost.

As part of the EC funded d-Liver project, the cost of hepatocytes derived from iPSCs was calculated, taking into consideration the most recent research, which compared drug metabolism activity in iPSC-derived hepatocytes with human hepatocytes [2]. In order to obtain the hepatocytes, the iPSCs in this publication required a four-stage differentiation protocol with a variety of recombinant growth factors. In addition, the cells were infected with adenovirus directed to over-express 2 transcription factors. Considering growth factors alone (without taking into account the cost of culture media, virus production, culture ware and the cost of failures), it was calculated that the cost to generate enough hepatocytes sufficient to populate a bioreactor capable of impacting on a patient with decompensating liver disease would be approximately £150,000 each time. With current technologies, it is unlikely that iPSC-derived hepatocytes will be a practical solution in extracorporeal liver devices. Widespread cost-effective use in other technologies - such as in routine in vitro toxicity testing – also remains a distant possibility.


And what about the cost of hepatocytes from stem cells (sufficient to provide sufficient function)

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<th>Amount in ng</th>
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**TOTAL**  
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If it was just dexamethasone

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**B-13 TOTAL**  
0.03
Current cost of iPSC-derived hepatocytes

- A human "B13-like" cell could offer a potential route to delivering a cost-effective, simple solution to the production of functional hepatocytes in vitro. The B-13 cell is a rat pancreatic acinar-like cell line that is readily expandable in simple culture medium and in response to one simple glucocorticoid hormone, differentiates into non-proliferative hepatocyte-like (B-13/H) cells. As part of a program to develop an human equivalent, this laboratory has investigated the biology of the B-13 cell. B-13 cells model a pathophysiological response of rodent and human acinar tissue to differentiate into hepatocytes both in vitro and in vivo on exposure to high levels of glucocorticoid [3,4]. The cells retain a degree of biological stability in that they have maintained a normal karyotype in terms of the number of chromosomes per cell (although with some cytogenetic abnormalities); retain a requirement for substratum attachment for growth; and an ability to selectively engraft into the pancreas and liver [5]. Within the liver, the B-13 cells differentiate into hepatocytes [5]. The intrinsic value of this type of cell line is that it offers an unlimited and reproducible supply of hepatocytes in vitro, without the requirement for tissue donors. In the context of generating enough hepatocytes sufficient to populate a bioreactor capable of impacting on a patient with decompensating liver disease, the cost with respect to hormonal addition (using dexamethasone) is estimated to be £0.03 each time. On this basis, the cost of generating hepatocytes via a B-13 type approach (based on hormonal requirements alone, many of the other costs would be similar) is approx. 5 million times cheaper than using iPSCs. Given both the simplicity and costs of a B-13 approach to hepatocyte generation, a human B-13 equivalent would have huge potential in both experimental studies (e.g. toxicity screening) and clinical applications (e.g. extracorporeal liver support).


Workpackage progress – B-13s

B-13s

- expandable
- simple differentiation (1 cheap hormone)
- reliable
- CHEAP
- Advantages - understanding some of the mechanisms