MICROENCAPSULATION AND DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS INTO FUNCTIONAL HEPATOCYTES

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ABSTRACT

The liver is the major organ responsible for detoxification of the gastrointestinal tract, metabolism of macromolecules, and homeostasis of various essential processes in mammalian systems. This organ is highly vascularized and receives about 25% of the cardiac output to perform its function [1]. The liver is composed of a variety of cells with the basic functional unit responsible for the liver's vital function called a hepatocyte. Hepatocytes are about 25 μm in diameter and account for approximately 75% of the liver volume [2]. These cells are quite active metabolically and perform functions such as glucose metabolism (glycolysis), synthetic synthesis of glucose (gluconeogenesis), fat metabolism, urea synthesis, and plasma protein secretion. From these facts alone, it can be stated that the failure of this organ can lead to chronic and fatal consequences thereafter.

An emerging strategy has been to develop bioartificial liver systems to combat the problem. The basic idea behind these methodologies is hemodialysis, or the filtering or blood plasma. A variety of artificial liver systems have been evaluated [3] and found to be inefficient for long term patency. Promising technologies, specifically bioartificial liver devices, seed hepatocytes (and other cells depending on the device) into a support structure that allows for the convective and diffusive transport of blood plasma needed for detoxification and other hepatic activities [4]. Many creative operational strategies and designs exist [5, 6, 7], however the rate-limiting step for the clinical benefit of these devices to be realized seems to be a reliable cell source.

To address this need our research team has recently focused our attention to the leaps and bounds that have been made in the field of stem cell biology. Stem cells by definition: (i) can recreate themselves in at least one daughter cell after cell division; and (ii) have stable developmental potential to form derivatives of several cell lineages. In vitro, stem cells are the cell source for newly forming or damaged tissue structures. There are many different types of stem cell populations that are responsible for different cell lineages such as hematopoietic, neural, and mesenchymal stem cells. Although each has been cultured in vitro successfully and the plasticity [11], or ability to form non-lineage cells from a certain stem cell, is a theory that holds much promise, these cells are quite difficult to isolate and purify and they are found in relatively low amounts in most mammals.

The final class of stem cells are embryonic stem cells (ESC) [12], which are derived from pre-implantation or peri-implantation embryos making them easy to isolate and culture. These cells are totipotent, meaning that they have the potential to be cultured for several passages and be coaxed into becoming cells of the cardiac [13], endothelial [14], neuronal [15], hematopoietic [16], and other cells from all three germ layers [17]. Because of these two features of simple isolations and totipotency, these cells provide a model system of stem cell efforts towards controlling differentiation. For this project in particular, certain groups have shown that embryonic stem cells can differentiate into functional hepatocytes with and without exogenous growth factors, which are cellular signaling molecules that promote differentiation [18, 19]. What is most interesting to our group is that in both cases, the ESC were cultured using a hanging drop technique. An in vitro hanging drop technique mimics in vivo cell morphology because normally, when ESC are allowed to differentiate in a suspension culture, they form spherical multi-cellular aggregates, termed embryoid bodies (EBs) [20]. This embryoid body allows for the cell-cell and cell-matrix interactions that are necessary for both self-renewal and differentiation of ESC. This structure/function relationship of a 3D spheroidal morphology/differentiation into functional hepatocytes, is the hypothesis that drives the research to be presented.

By mimicking this morphology, while keeping in mind future applications of the work, we hope to precisely control embryonic stem cell differentiation into functional hepatocytes in a characterized microenvironment, which is the main objective of this design project. The work presented has direct, scale-up capability to an automated stem cell bioreactor and also the cryopreservation of microencapsulated embryoid bodies for inventory purposes of such a bioreactor.

The hypothesis of the design is that microencapsulation of ESC can provide the microenvironment for embryoid body formation and proper mechanotransduction stimulus for hepatocyte differentiation (assuming that these transduction pathways, implicit in the embryoid body, are the reason for hepatocyte differentiation), as well as proper conditions for growth and maintenance of the cells. Microencapsulation, in short, is the entrapment (and even protection) of a biological, either molecular or cellular, for purposes of transport, delivery, and/or recovery of the biological for later purposes. The main objective of microencapsulation in the past has been to protect cells of interest from the host response to an implanted device, such as...
pancreatic islet cells for diabetes treatment [21]. Encapsulated cell technology has also branched out to the therapies and diseases ranging from Parkinson's disease [22] to hypocalcemia [23] and even fulminant hepatic failure [24]. Many encapsulation systems exist, and each have specific applications as well as their own pros and cons. In our design microencapsulation will be accomplished using alginate, a natural, polymer composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G), and poly-L-lysine constructs [25]. Alginate/PLL has proved to provide greater cell densities, mechanical stability, suspension culture and facile manipulation of mammalian cells. Furthermore, alginate/PLL systems are the most matured of all encapsulation methods and have withstood the test of time and experimentation, which has developed a rigorously characterized and tested polymer system. The microcapsule formation is a polymerization reaction that chemically reversible, therefore allowing for recovery of the lineage cell type after differentiation.

The variables of major concern in most encapsulation efforts are the capsule diameter and the molecular weight cut-off, i.e. the size constraint of diffusible particles into and out of the material. For the work presented models have been developed to optimize these variables to ensure adequate transport of oxygen, small metabolites, and growth factor delivery to, as well as the size constraint of diffusible particles into and out of the material. For the work presented models have been developed to optimize these variables to ensure adequate transport of oxygen, small metabolites, and growth factor delivery to, as well as waste removal from, cells. The molecular weight cut-off and diameter are variables that can be manipulated chemically and technically, respectively. The molecular weight cut-off has been shown to be a function of contact time, temperature, and concentration of poly-L-lysine with the alginate bead [26]. The diameter of the microcapsule has been controlled by many different droplet formation schemes [27]. Out of the variety of droplet formation mechanism (Fig. 1), an electrostatic droplet formation devices has shown the most promising data of demonstrating precise control in an easily operated apparatus [28].

REFERENCES


