MICROENCAPSULATION AND DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS INTO FUNCTIONAL HEPATOCYTES

Parekkadan B, Maguire T, Schloss R, Yarmush ML

Department of Biomedical Engineering, Rutgers University, Piscataway, New Jersey 08854

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 (glycogenolysis), 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. Principally, two types of liver failure account for 30,000 deaths each year in the United States alone[http://www.emedicine.com]. Cirrhosis of the liver, commonly caused by alcoholism or viral infections, occurs when fibrotic tissue materializes in place of damaged liver tissue, impeding the unique ability of the liver to regenerate. Fulminant hepatic failure is a rapidly progressing disease, caused by similar means, that can deteriorate the liver within weeks of onset. Transplantation has been the only viable option for clinicians, yet many patients die due to the limitations of available liver donors.

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 founded 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 vivo*, 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 hematopoeitic, neural, and mesenchymal stem cells. Although each has been cultured *in vitro* [8,9,10] 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 microencapsualted 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, ie. 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

Guyten, AC. Textbook of medical physiology, 8 ed. Philadelphia,PA : W.B. Saunders, 1991. p186.

Fournier, RL. Basic Transport Phenomena in Biomedical Engineering. Philadelphia, PA: Taylor & Francis, 1999.

Yarmush, ML, Dunn JC, Tompkins RG (1992). Assessment of artificial liver support technology. Cell Transplant. 1: 323-341.

4. Jauregui HO, Mullon CJ, Solomon BA. "Extracorporeal Artificial Liver Support". Principles of Tissue Engineering. Boulder,CO: R.G. Landes, 1997.

5. Nyberg SL, Shatford RA, Peshwa MV, White JG, Cerra FB, Hu W-S (1993). Evaluation of a hepatocyte entrapment hollow fiber bioreactor: a potential bioartificial liver. Biotechnol.Bioeng. 41: 194-203.

6. Kelly JH, Sussman NL (1994). The Hepatix extracorporeal liver assist device in the treatment of fulminant hepatic failure. ASAIO J. 40: 83-85.

7. Roy P, Baskaran H, Tilles AW, Yarmush ML, Toner M (2001). Analysis of oxygen transport to hepatocytes in a flat-plate microchannel bioreactor. Ann Biomed Eng 29:947-55.

8. Fraser CC, Szilvassy SJ, Eaves CJ, Humphries RK (1992). Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability. Proc Natl Acad Sci U S A 89:1968-72.

9. Reynolds BA, Weiss S (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 225: 1707-1710.

10. Friednestein AJ, Gorskaja JF, Kulagina NN (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp. Hematol. 4: 267-274.

11. Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL (1999). Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science 283: 534-537.

12. Evans MJ, Kaufman MH (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature 292: 154-165.

13. Rohwedel J, Maltsev V, Bober E, Arnold HH, Hescheler J, Wobus AM(1994). Muscle cell differentiation of embryonic stem cells reflects myogenesis in vivo: developmentally regulated expression of myogenic determination genes and functional expression of ionic currents. Dev. Biol. 164: 87-101.

14. Wang R, Clark R, Bautch VL (1992). Embryonic stem cell-derived cystic embryoid bodies form vascular channels: an in vitro model of blood vessel development. Development 114: 303-316.

15. Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI (1995). Embryonic stem cells express neuronal properties in vitro. Dev. Biol. 168: 342-357.

16. Nagy A, Gocza E, Nagy R, Abramow-Newerly W, Roder JC (1993). Derivation of completely cell culture derived mice from early-passage embryonic stem cells. Proc. Natl. Acad. Sci.USA 90: 8424-28.

17. Itskovitz-Eldor J et al. (2000). Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol. Med. 6: 88-95.

18. Chinzei R, Tanaka Y, Shimizu-Saito K, Hara Y, Kakinuma S, Watanabe M, Teramoto K, Arii S, Takase K, Sato C, Terada N, Teraoka H (2002).Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. Hepatology 36:22-29.

19. Yamada T, Yoshikawa M, Kanda S, Kato Y, Nakajima Y, Ishizaka S, Tsunoda Y (2002) .In vitro differentiation of embryonic stem cells into hepatocyte-like cells identified by cellular uptake of indocyanine green. Stem Cells 20:146-54.

20. Keller GM (1995). In vitro differentiation of embryonic stem cells. Curr Opin Cell Biol 7:862-869.

21. Lanza, RP, Hayes JL, Chick WL. Encapsulated cell technology. NatureBiotechnol. 14:1107-1111.

22.   Winn SR, Tresco PA, Zielinski B, Green LA, Jaeger CB, Aebischer P. Behavioral recovery following intrastriatal implantation of microencapsulated PC12 cells (1991). *Exp. Neurol.* **113:** 322.

23.   Fu XW, Sun AM (1989). Microencapsulated parathryoid cells as a bioartificial parathyroid. *Transplantation* **47:** 432

24.   Sun AM, Cai Z, Shi Z, Ma F, O'Shea GM, Gharapetian H (1986). Microencapsualted hepatocytes as a bioartificial liver. *Trans. Am. Soc. Artif. Intern. Organs* **32:** 39.

25.   Lim F, Sun AM (1980). Microencapsulated islets as bioartificial endocrine pancreas. *Science*   **210:** 980.

26,   Vandenbossche GMR, Van Oostveldt P, Demeester J, Remon JP (1993). Themolecular weight cut-off of microcapsules is determined by the reaction between alginate and polylysine. *Biotechnol. Bioeng.* **42:** 381-386.

27.   Goosen MF. Fundamentals of Animal Cell Encapsulation and Immobilization. CRC Press:Ann Arbor, 1993.

28.   Al-Hajry HA, Al-Maskry SA, Al-Kharousi LM, El-Mardi O, Shayya WH, Goosen MF (1999). Electrostatic encapsulation and growth of plant cell cultures in alginate.*Biotechnol Prog* **15:**768-74.