

THREE-DIMENSIONAL LIVER CELL AND TISSUE CULTURE SYSTEM

This is a division of application Ser. No. 08/241,259, filed May 11, 1994, now U.S. Pat. No. 5,483,988; which is a continuation-in-part of Ser. No. 08/131,361, filed Oct. 4, 1993, now U.S. Pat. No. 5,443,950; which is a division of Ser. No. 07/575,518, filed Aug. 30, 1990, U.S. Pat. No. 5,266,480; which is a division of Ser. No. 07/402,104, filed Sep. 1, 1989, U.S. Pat. No. 5,032,508; which is a continuation-in-part of Ser. No. 07/242,096, filed Sep. 8, 1988, U.S. Pat. No. 4,963,489; which is a continuation-in-part of Ser. No. 07/038, 110, filed Apr. 14, 1987, abandoned; which is a continuation-in-part of 07/036,154, filed Apr. 3, 1987, U.S. Pat. No. 4,721,096 which is a continuation of Ser. No. 06/853,569, filed Apr. 18, 1986, abandoned; each of which is incorporated by reference herein in its entirety.

1. INTRODUCTION

The present invention relates to a three-dimensional cell and tissue culture system. In particular, it relates to this culture system for the long term culture of liver cells and tissues *in vitro* in an environment that more closely approximates that found *in vivo*. The culture system described herein provides for proliferation and appropriate liver cell maturation to form structures analogous to tissue counterparts *in vivo*. The resulting liver tissues survive for prolonged periods, perform liver-specific functions, and maintain hepatic tissue architecture following *in vivo* implantation.

The liver cultures have a variety of applications ranging from transplantation or implantation *in vivo*, to screening cytotoxic compounds and pharmaceutical compounds *in vitro*, to the production of biologically active molecules in "bioreactors" and to the construction of extracorporeal liver assist device.

2. BACKGROUND OF THE INVENTION

The liver is a dynamic organ that plays an important role in a variety of physiological processes. The complex functions of the liver include metabolism, storage, excretion, secretion of plasma proteins such as albumin and detoxification of harmful substances by enzymes of the cytochrome P-450 system. In addition, the usually quiescent liver is also capable of remarkable mitotic activities under certain circumstances. The major cell population of the liver is the parenchymal cells (PC), also known as hepatocytes. The liver also contains several other cell types such as endothelial cells, adipocytes, fibroblastic cells and Kupffer cells, collectively referred to as stromal (littoral) cells.

2.1 LIVER CELL CULTURES

In an attempt to study the diverse liver functions and the cell types responsible therefor, *in vitro* cultures of liver cells have been prepared from humans as well as from experimental animals. Primary cultures of rat hepatocytes have been used extensively to study the effects of potential toxins on enzyme leakage, metabolism, and cellular membranes (Grisham, 1979, *Int. Rev. Exp. Pathol.* 20:123-210; Acosta and Mitchell, 1981, *Biochem. Pharmacol.* 30:3225-3230). However, such culture systems have a number of drawbacks, and none have provided for the proliferation of liver PC.

In vitro, adult hepatocytes proliferate for only short time periods, although their ability to produce albumin and display cytochrome P-450 enzyme activity may be prolonged if

they are co-cultured with other liver-derived extracellular matrix (ECM) substances or with certain combinations thereof. In liquid culture, the viability of hepatocytes and the ability of these cells to manifest inducible cytochrome P-450 enzyme activity decline as a function of time (Sirica and Pitot, 1980, *Pharmacol. Rev.* 31:205-228). In addition, cell division usually is limited to the first 24-48 hr of culture (Clayton and Darnell, 1983, *Mol. Cell Biol.* 3:1552-1561; Chapman et al., 1973, *J. Cell Biol.* 59:735-747). The viability of adherent hepatocytes in monolayer cultures persists for somewhat longer periods but specialized activity is also lost rapidly (Deschenes et al., 1980, *In Vitro* 16:722-730).

Towards the goal of enhancing hepatocyte growth and prolonging liver-specific functions *in vitro*, hepatic cells have been cultured on various matrices including type I collagen plates and membranes (Michalopoulos and Pitot, 1975, *Exp. Cell Res.* 94:70-78), homogenized liver biomatrix (Reid et al., 1980, *Ann. N.Y. Acad. Sci.* 349:70-76), in collagen type IV or laminin-rich gels (Bissell et al., 1987, *J. Clin. Invest.* 79:801-812), sandwiched between two layers of type I collagen (Dunn et al., 1989, *FASEB J.* 3:174-177), and on plates coated with fibronectin or the other extracellular matrix proteins (Deschenes et al., 1980, *In Vitro* 16:722-730). All of these methods have been reported to extend the functional life of hepatocytes *in vitro* to some extent.

Substantial improvements in this regard were produced by culturing-PC with various types of non-parenchymal stromal or littoral hepatic cells or non-hepatic stromal cells. Both human and rat hepatocytes which were co-cultured with liver endothelial cells of the same species maintained specific functions for weeks in culture, although they did not undergo a significant expansion in numbers (Guguen-Guillouzo, et al., 1983, *Exp. Cell Res.* 143:47-54; Begue et al., 1983, *Biochem. Pharmacol.* 32:1643-1646). Rat hepatocytes which were co-cultured with human fibroblasts (Kuri-Harcuch and Mendoza-Figueroa, 1989, *Differentiation* 41:148-157) and endothelial cells (Begue et al., 1983, *Biochem. Pharmacol.* 32:1643-1646) were reported to sustain cytochrome P-450 activity for more than 10 days. Thus, these mixed hepatocyte co-culture systems may provide microenvironments similar to those *in vivo* by optimizing cell-cell interactions. In addition, various PC functions may be regulated and/or optimized by other hepatic cells. For example, Kupffer cell secretory products have been reported to modulate PC cytochrome P-450 enzyme activity (Peterson and Renton, 1984, *J. Pharmacol. Exp. Ther.* 229:299-304). The attachment of PC to fibroblasts is evidently contingent upon the secretion of specialized extracellular matrix substances by Kupffer cells (Michalopoulos et al., 1979, *In Vitro* 15:769-806). Hepatic endothelial cells also may produce important components of the extracellular matrix (Guguen-Guillouzo, et al., 1983, *Exp. Cell Res.* 143:47-54), and adipocytes may provide the requisite raw materials for the renewal of cell membranes in metabolically-active hepatocytes.

Although the viability and functional activities of cultured hepatic PC can be prolonged *in vitro* if the cells are co-cultured with non-parenchymal liver stromal cells, support cells from other tissues, or their secretory products, PC proliferation is limited or absent in these systems. Mitoses in co-cultures of hepatic cells have been ascribed primarily to non-parenchymal elements (Guguen-Guillouzo, et al., 1983, *Exp. Cell Res.* 143:47-54). Several reports indicate that non-parenchymal liver cells may express functions similar to hepatocytes (Grisham, 1980, *Ann. N.Y. Acad. Sci.* 349:128-137) although the nature of these non-PC has not been unequivocally established.