TEXAS MEDICAL CENTER NASA/HOHNSON SPACE CENTER
COOPERATIVE AGREEMENT PROGRAM NCC 9-36, ROUND II

COVER SHEET FOR FINAL REPORT

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Name of Subcontractor: Boris Yoffe, M.D.

Title: Associate Professor of Medicine and Molecular Virology

Institute: Baylor College of Medicine

Name of Project: Three Dimensional Primary hepatocyte Culture.

Amount of Grant: [redacted]

* Amount spent, if different from Amount Granted: [redacted]

Date Project was completed: March 31, 1998

Grants Officer: Thomas E. Wilson

Title: Director of Sponsored Programs

Phone: (713) 798-6970

Fax: (713) 798-6990

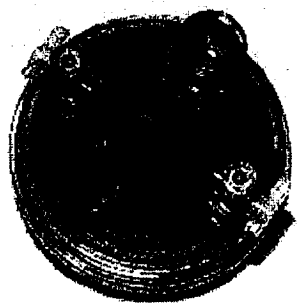
FINAL REPORT

For TMC/NASA-JSC cooperative agreement NCC 9-36 entitled "**Three Dimensional Primary hepatocyte Culture**"

Please find enclosed final report regarding work accomplished during Round II of our funding. Observations from our preliminary results were recently presented at the FASEB (Federation of American Societies for Experimental Biology) Summer Research Conference titled "Mechanism of Liver Growth and Differentiation in Health and Disease" (July 11-16 1998 at Snowmass Village, Colorado) and at COSPAR (Committee on Space Research) meeting in Nagoya, Japan. Also, the manuscript summarizing these results has been accepted for publication in *Advances in Space Research.*

1) Our results demonstrated for the first time the feasibility of culturing PHH in microgravity bioreactors that exceeded the longest period obtained using other methods. Within the first week of culture, isolated hepatocytes started to form aggregates, which continuously increased in size (up to 1 cm) and macroscopically appeared as a multidimensional tissue-like assembly. To improve oxygenation and nutrition within the spheroids we performed experiments with the biodegradable nonwoven fiber-based polymers made from polyglycolic acid (PGA). It has been shown that PGA scaffolds stimulate isolated cells to regenerate tissue with defined sizes and shapes and are currently being studied for various tissue-engineering applications. Our data demonstrated that culturing hepatocytes in the presence of PGA scaffolds resulted in more efficient cell assembly and formations of larger cell spheroids (up to 3cm in length, see figure). The histology of cell aggregates cultured with PGA showed polymer fibers with attached hepatocytes.

2) We initiated experiments to co-culture primary human hepatocytes with human microvascular endothelial cells in the bioreactor. The presence of endothelial cells in co-cultures were established by immunohistochemistry using anti-CD34 monoclonal Ab. Our preliminary data demonstrated that cultures of purified hepatocytes with human microvascular endothelial cells exhibited better growth and expressed higher levels of albumin mRNA for a longer period of time than cultures of purified primary human hepatocytes cultured alone.



3) We also evaluated microsomal deethylation activity of hepatocytes cultured in the presence of endothelial cells. We assessed metabolites of midazolam, a water-soluble benzodiazepine that is

rapidly metabolized via cytochrome P450, by gas chromatography. We observed that co-cultures of human hepatocytes with human microvascular endothelial cells contain higher levels of midazolam metabolites than hepatocytes that were cultured alone. These findings correlate with the previous data that demonstrated that the cultures of hepatocytes with endothelial cells expressed higher levels of albumin mRNA for a longer period of time. The clearance of midazolam and appearance of its metabolites in culture media of hepatocytes co-cultured with endothelial cells indicate that endothelial cells play an important role in supporting functional activity of primary hepatocytes in culture. No microsomal activity was observed in hepatocytes cultured by conventional method.

In summary, we have established liver cell culture, which mimicked the structure and function of the parent tissue. We believe that these studies may lead to the establishment of a cell culture system, which will be utilized in the development of hepatic support systems, gene therapy, and hepatocyte transplantation.

For TMC/NASA-JSC cooperative agreement NCC 9-36 entitled "Three Dimensional Primary Hepatocyte Culture"

The work accomplished for this project was presented in the FASEB (Federation of American Societies for Experimental Biology) Summer Research Conference titled "Mechanism of Liver Growth and Differentiation in Health and Disease" (July 11-16 1998 at Snowmass Village, Colorado).

Abstract:

INDUCTION OF THREE-DIMENSIONAL GROWTH OF HUMAN LIVER CELLS IN SIMULATED MICROGRAVITY.

V.I. Khaoustov(1)., G.J. Darlington(1), H.E. Soriano (1), B. Krishnan(1), D. Risin(2), N.R. Pellis(2), B. Yoffe(1)
(1) Baylor College of Medicine, (2) NASA/JSC & Wyle Laboratories, USA.

The establishment of long-term cultures of functional primary human liver cells (PHLC) is formidable. To achieve this goal, hepatoblastoma cells (HepG2) and PHLC were cultured in a bioreactor under simulated microgravity conditions. Bioreactors simulate microgravity by creating a unique environment with low shear force and high-mass transfer. HepG2 and PHLC aggregates were readily formed and increased in size up to 1 cm in length. The expansion of liver cell cultures in bioreactors was further evaluated using microcarriers and biodegradable scaffolds. While microcarriers did not affect cell growth, PHLC cultured with biodegradable scaffolds formed aggregates up to 3 cm in length. Analyses of PHLC spheroids revealed tissue-like structures comprised of hepatocytes, biliary epithelial cells and/or progenitor liver cells that were arranged as bile duct-like structures along nascent vascular sprouts. Electron microscopy revealed groups of cohesive hepatocytes and bile canaliculi with multiple microvilli and tight cellular junctions. Hepatocytes were further organized into tight clusters surrounded by complex stromal structures and reticulin fibers. Moreover, albumin mRNA was expressed throughout the 60-day culture. In summary, we have shown for the first time that a simulated microgravity environment is conducive for maintaining long-term cultures of functional hepatocytes. This model system will facilitate studies of liver regeneration and cell-to-cell interactions that occur *in vivo*.