



Microgravity-Enhanced Stem Cell Selection

This method provides rapid selection and proliferation of stem cells using a hydrofocusing bioreactor.

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Stem cells, both embryonic and adult, promise to revolutionize the practice of medicine in the future. In order to realize this potential, a number of hurdles must be overcome. Most importantly, the signaling mechanisms necessary to control the differentiation of stem cells into tissues of interest remain to be elucidated, and much of the present research on stem cells is focused on this goal. Nevertheless, it will also be essential to achieve large-scale expansion and, in many cases, assemble cells in 3D as transplantable tissues. To this end, microgravity analog bioreactors can play a significant role.

Microgravity bioreactors were originally conceived as a tool to study the cellular responses to microgravity. However, the technology can address some of the shortcomings of conventional cell culture systems; namely, the deficiency of mass transport in static culture and high mechanical shear forces in stirred systems. Unexpectedly, the conditions created in the vessel were ideal for 3D cell culture. Recently, investigators have demonstrated the capability of the microgravity bioreactors to expand hematopoietic stem cells compared to

static culture, and facilitate the differentiation of umbilical cord stem cells into 3D liver aggregates.

Stem cells are capable of differentiating into functional cells. However, there are no reliable methods to induce the stem cells to form specific cells or to gain enough cells for transplantation, which limits their application in clinical therapy. The aim of this study is to select the best experimental setup to reach high proliferation levels by culturing these cells in a microgravity-based bioreactor. In typical cell culture, the cells sediment to the bottom surface of their container and propagate as a one-cell-layer sheet. Prevention of such sedimentation affords the freedom for self-assembly and the propagation of 3D tissue arrays.

Suspension of cells is easily achievable using stirred technologies. Unfortunately, in conventional bioreactors, stirring invokes deleterious forces that disrupt cell aggregation and results in cell death. First-generation rotating bioreactors provided rotation on the horizontal axis, which resulted in the suspension of cells without stirring, thus providing a suitable environment to propagate cells without sedimentation to a surface. The rotating-

wall bioreactors did not provide a way to remove air bubbles that were causing shear and disrupting 3D cultures. Johnson Space Center successfully engineered the hydrofocusing bioreactor (HFB) that resolved the problem of removing the air bubbles from the fluid medium of NASA's rotating-wall space bioreactors.

The HFB uses the principle of hydrodynamic focusing that simultaneously produces a low-shear fluid culture environment and a variable hydrofocusing force that can control the movement, location, and removal of suspended cells, tissues, and air bubbles from the bioreactor. The HFB is a rotating, dome-shaped cell culture vessel with a centrally located sampling port and an internal viscous spinner. The vessel and spinner can rotate at different speeds either in the same or opposite directions. Rotation of the vessel and viscous interaction at the spinner generate a hydrofocusing force. Adjusting the differential rotation rate between vessel and spinner controls the magnitude of the force.

This work was done by Pier Paolo Claudio and Jagan Valluri of Goddard Space Flight Center. Further information is contained in a TSP (see page 1). GSC-15807-1

Diagnosis and Treatment of Neurological Disorders by Millimeter-Wave Stimulation

These techniques enable new treatments for neurological disorders and dysfunction.

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Increasingly, millimeter waves are being employed for telecomm, radar, and imaging applications. To date in the U.S, however, very few investigations on the impact of this radiation on biological systems at the cellular level have been undertaken. In the beginning, to examine the impact of millimeter waves on cellular processes, researchers discovered that cell membrane depolarization may be triggered by low levels of in-

tegrated power at these high frequencies. Such a situation could be used to advantage in the direct stimulation of neuronal cells for applications in neuroprosthetics and diagnosing or treating neurological disorders.

An experimental system was set up to directly monitor cell response on exposure to continuous-wave, fixed-frequency, millimeter-wave radiation at low and modest power levels (0.1 to

100 safe exposure standards) between 50 and 100 GHz. Two immortalized cell lines derived from lung and neuronal tissue were transfected with green fluorescent protein (GFP) that locates on the inside of the cell membrane lipid bi-layer. Oxonol dye was added to the cell medium. When membrane depolarization occurs, the oxonol bound to the outer wall of the lipid bi-layer can penetrate close to the inner wall where

the GFP resides. Under fluorescent excitation (488 nm), the normally green GFP (520 nm) optical signal quenches and gives rise to a red output when the oxonol comes close enough to the GFP to excite a fluorescence resonance energy transfer (FRET) with an output at 620 nm.

The presence of a strong FRET signature upon exposures of 30 seconds to 2 minutes at 5–10 mW/cm² RF power at 50 GHz, followed by a return to the normal 520-nm GFP signal after a few minutes indicating repolarization of the membrane, indicates that low levels of RF energy may be able to trigger non-destructive membrane depolarization without direct cell contact. Such a mechanism could be used to stimulate neuronal cells in the cortex without the need for invasive electrodes as millimeter waves penetrate skin and bone on the order of 1–5

mm in depth. Although 50 GHz could not readily penetrate from the outer skull to the center of the cortex, implants on the outer skull or even on the scalp could reach the outer layer of the cerebral cortex where substantial benefit could be realized from such non-contact type excitation.

The stimulation system described here for cerebral cortex, brainstem, spinal cord, or peripheral nerves includes an implantable housing, a control unit carried by the implantable housing, a millimeter-wave delivery device (including at least one emission site), and at least one millimeter-wave source operatively coupled to the control unit, and coupled to at least one millimeter-wave emission site. Optional components for monitoring of neuronal function can be included, such as an electroencephalographic system, an

electromyographic system, a system for optical or infrared imaging of intrinsic neuronal signals, and/or magnetic resonance imaging/spectroscopy systems.

This work was done by Peter H. Siegel of Caltech and Victor Pikov of HMRI for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

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