New Technological Advances in Stem Cell Research

Several other companies also are developing new approaches to culturing or utilizing stem cells for various applications. In a series of recent poster presentations, scientists from Plasticell, Celartia, and Cellular Dynamics International provided details and data on how each company's technology was being employed to advance stem cell research.

Turning hESCs into Functional Hepatocytes

Plasticell has developed the Combinatorial Cell Culture (CombiCult®) platform—a high-throughput technology specifically designed for the discovery of novel stem cell differentiation protocols.

The way it works is that stem cells on beads are exposed to multiple combinations of media, containing active agents such as growth factors or small molecules. A unique tagging system allows the cell culture history of each bead to be determined, and tens of thousands of media combinations can be tested in each screen. The goal is to discover the optimal combinations for effective stem cell differentiation.

A disposable split-pool device that facilitates bead manipulation and a custom developed bioinformatics software program, Ariadne™, for protocol selection have further streamlined the CombiCult system. The platform reportedly has been successfully used to discover novel differentiation protocols for many different starting stem cell types and differentiated progeny, e.g., hepatocytes, neurons, cardiomyocytes, and osteoblasts from human and mouse embryonic and mesenchymal stem cells.

According to Plasticell, since large numbers of conditions can be tested in each screen it is possible to efficiently discover optimal protocols that have advantages over more traditional cell culture methods.

For example, in a poster entitled "Directed differentiation of human embryonic stem (hES) cells into functional hepatocytes using the combinatorial cell culture platform CombiCult," Jeyakumar et al., from Plasticell describe a method for identifying novel serum-free protocols for generating hepatocytes from human pluripotent stem cells.

In this CombiCult screen, hES cells cultured on microcarrier beads were shuffled through 3,375 combinations of cell culture media, containing growth factors and/or small molecules over a 21-day period. Immunostaining for albumin and CYP3A4 expression was used to identify beads bearing differentiated cells.

These positive "hit" beads were identified and sorted using a large particle flow sorter (COPAS) and the tags attached to the beads analyzed, enabling identification of hepatic differentiation protocols.

The researchers reported the discovery of a number of efficient serum-free differentiation protocols. These protocols were further validated in a monolayer culture system, and the cells characterized at phenotypic and functional levels. Specifically, they demonstrated that hepatic cells generated using CombiCult-discovered protocols: 1) exhibit a polygonal shape resembling hepatic morphology, 2) express hepatic markers such as CYP3A4, albumin, and FoxA2, and 3) illustrate functional characteristics of hepatocytes such as glycogen storage and AcLDL uptake.

Such hepatic-like cells could serve as "a valuable tool for regenerative medicine and drug discovery applications," wrote the scientists.

Stem Cell Culture in Petaka

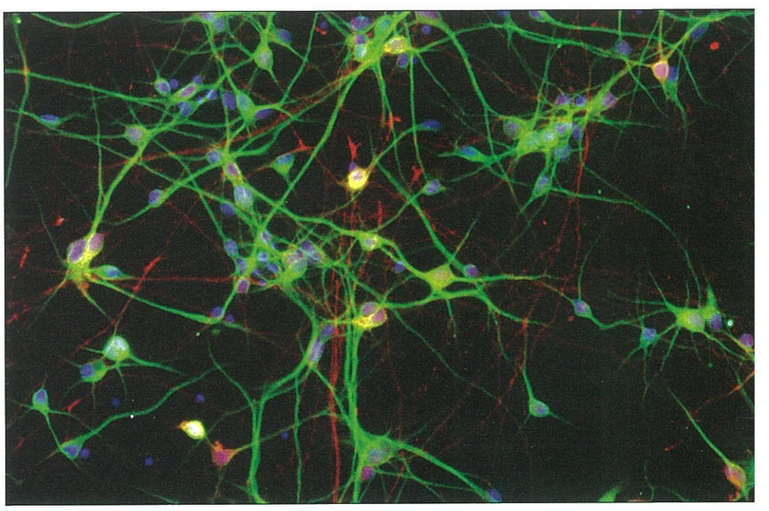
Celartia has developed a solid, closed, autonomous, and disposable cell culture device to provide cultured cells with a stable oxygen supply within the physiologic limits, independent from additional CO_2 for balancing the pH, and not requiring ambient saturated humidity for controlling dehydration. The company designed the Petaka® G3 for use with cultures of primary cells, cell lines, and both adult and embryonic stem cells.

The heart of this device is the Duct Respiratory Chamber that integrates a 0.2 micron pore filter protected vent connected to the culture chamber through a 38.6 inch long microgas channel that acts as an automatic valve. This configuration allows oxygen diffusion into the culture only when the outside O_2 partial pressure is higher than the internal O_2 partial pressure (see GEN, November 1, 2012, p. 46).

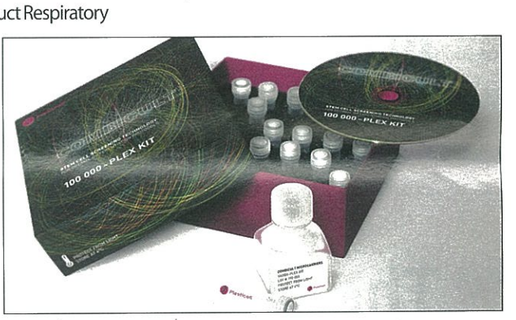
In a poster entitled "Stem cell culture in Petaka, devices with autonomously regulated hypoxia," Vicente et al., from Celartia, reported the results of an experiment to demonstrate the persistence of pluripotent cell markers and early differentiation markers, studied at the level of RNA expression. According to the research team, human embryonic stem cells cultured in Petaka and in parallel in regular 6-well plates were both tested for Oct3/4, Sox7, Brachury, and Meox1 expression. In stem cell research all four of these transcription factors are used as indicators of primitive state stem cells or as pluripotency markers.

Cells in hypoxia environment (Petaka) grew faster and reached high confluency in 4 days compared with 75% confluency in 6-well plates, noted the scientists. Cells in Petaka showed the same morphology and consistent expression of markers with differences in intensity.

Cells in PetakaG3 in hypoxia at confluent level showed (1) decrease of Oct3/4 expression (Ratio Petaka/plate = 0.16), (2) same levels of Sox 7 expression (Ratio Petaka/plate = 1.06), (3) superior levels of Brachury expression (Ratio Petaka/plate = 2.51), and (4) superior levels of Meox1 expression (Ratio Petaka/plate = 2.59), the Celartia group reported.



Human iPS cell derived iCell® Neurons Green = MAP2 (microtubule-associated protein 2); Red = GABA (gamma-Aminobutyric acid).
Cellular Dynamics International

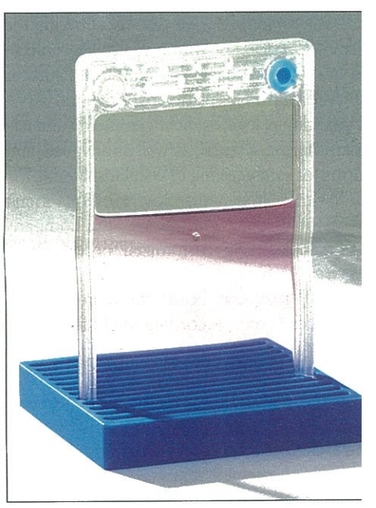


Plasticell says its bead-based CombiCult combinatorial screening technology allows scientists to discover new and improved stem cell differentiation protocols.

The scientists point out that this autonomous bioreactor technology can efficiently expand stem cells, while maintaining their undifferentiated traits. This facilitates the management of the stem cell cultures in regular atmosphere, thus increasing the spectrum of clinical applications and, consequently, opening a broad avenue for stem cell culture automation under safe GMP conditions, they explain.

Generating Human Forebrain Neurons

In a poster entitled "Applications of Human iPSC-derived Neurons using High Content Image-based Assays," Lucas Chase, group leader at **Cellular Dynamics International** (CDI), and colleagues from CDI and **Molecular Devices** report the development of a scalable protocol to generate cryopreserved human forebrain neurons from a normal human background. This commercialized product (iCell® Neurons) is a highly purified (≥ 90%) population of differentiated human neurons composed predominantly of GABAergic and glutamatergic subtypes.



Celartia's virtually hermetically enclosed cell culture chamber avoids direct contact of the cell environment with the atmosphere.

The poster described several applications fo iCell Neurons in high-content imaging assay including an optimized assay combining neurit outgrowth with live/dead cell analysis. High-re olution acquisition was performed using the In ageXpress® Micro or ImageXpress Micro XL sy tems and analyzed using the MetaXpress® sof ware. Using iCell Neurons, the scientists demor strated the capacity to use this system as a tool t test for modulators of neural outgrowth and ce survival as a means to test for neurotoxic effec of compounds and environmental agents.

The researchers also describe the develop ment of a high-content image-based assa to monitor synapse formation through th detection of presynaptic proteins. In adc tion, they demonstrated the maintenance synaptic network development and neuri outgrowth using an iCell neuron-astrocyte c culture system, when compared to a neuro only culture.