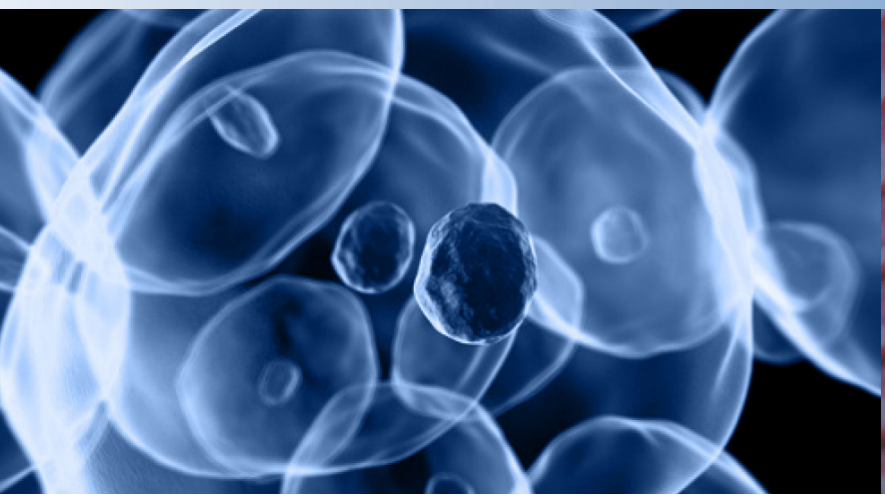
Case Study

Optimized cell culture: for bench and beyond



An ongoing demand for novel biopharmaceuticals, the increasing complexity of the production of biologics and a need for larger production capacity are all factors that have applied pressure to the cell culture process and its associated technologies. Cell culturing and its resources have evolved and scientists now work across numerous transformation established cell lines, primary cells and uniquely demanding cell types (e.g. stem cells) which is creating new challenges for bench and commercial scale processes. As such, the application of validated cell culture systems is essential for the safe, efficient production of monoclonal antibodies, therapeutic proteins, drugs and vaccines.

While much of the initial basic cell culture science takes place in the research lab, this work often needs to be scaled up in order to accommodate commercial requirements including process optimization and validation. Scaling up cell culture processes can prove difficult for many organizations as there are numerous regulatory and technological considerations, GMP compliance and the ever-present need to guard and prevent batch variability and contamination. A significant amount of time and effort goes into early stage research and it is therefore imperative that the transition from bench to industrial scale be an obvious and natural extension of the bench top cultivation conditions.

The development of the Thermo Scientific™ Nunc™ Cell Factory™ system has been driven by the need to support the commercialization of cell culture processes with a simple and effective solution. The Cell Factory system is a multi-layered solution designed for the cultivation of adherent cells ranging in scales: from a 1–2 layer system ideal for research needs, up to 40 layers for industrial scale culture, providing up to 25,280 cm^2 of cell culture surface area.

The Thermo Scientific™ Nunclon™ Delta certified cell culture surface offers consistent cell culture performance from layer to layer across multiple formats. All Cell Factory systems are certified to a Sterility Assurance Level (SAL) of 10^{-6} achieved following ISO 11137-2 guidelines.

Thermo SCIENTIFIC

Consistency from top to bottom

Traditional multi-layered cell culture systems have been used in the production of vaccines, recombinant proteins and the generation of cell mass where investigators hope to maximize output while keeping the footprint to a minimum. However, there has been concern of a possible lack of consistency in the quality of culture between layers resulting from the fact that it is challenging to view the culture conditions within the middle layers.

To address this presumption, a study was initiated to assess the uniformity of conditions and the consistency of cell culture in each layer of the Cell Factory system (Staggert *et al*. 2013). Four cell lines were cultured in a 10-layer Cell Factory system (CF10): Chinese Hamster Ovary (CHO), VERO, Madin-Darby Bovine Kidney (MDBK) and Madin-Darby Canine Kidney (MDCK). Cells were harvested from a source and diluted in prepared media to 30,000 cells/ml (CHO, MDBK, VERO) or 45,000 cells/ml (MDCK). These cells were incubated at 37°C without CO_2 control before being stained with crystal violet and imaged to analyze confluence and consistency.

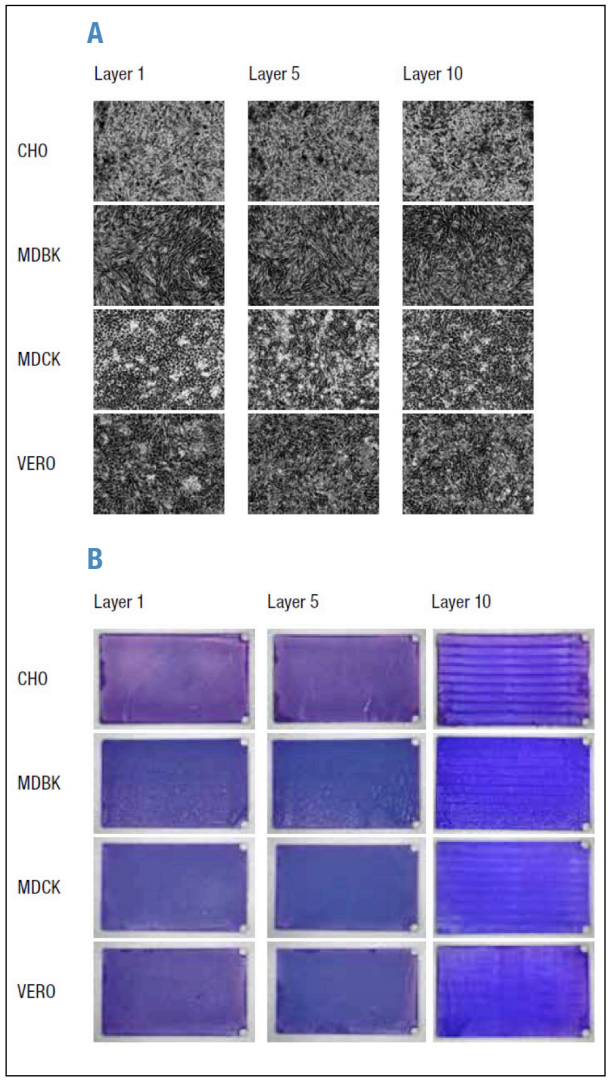
Comparison of the images showed a high degree of consistency over the surfaces and between layers (figure 1A), excluding layer 10 (figure 1B) on account of this layer being in contact with the incubators – a pattern commonly seen in cell culture. Images of individual layers reveal that within the CF10 there are no significant differences between layers in terms of cell growth, density and morphology.

Production of viral gene transfer vectors

Recombinant viruses are exceedingly efficient gene transfer vehicles and hold great value for use in protein expression or gene knockout experiments. Unlike other genetic targeting strategies, the use of viral vectors, based on the lentivirus (LV) or adeno-associated virus (AAV) for example, do not rely upon the utilization of transgenic animal models. Viral vectors are first fabricated in cell cultures before being transfected with the plasmid DNA of interest.

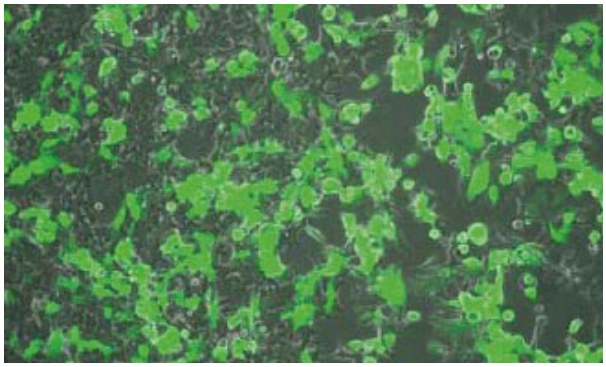
In order to cultivate a large number of cells and assess the growth kinetics of packaging cells used for the generation of recombinant viral vectors in the Cell Factory system, researchers made use of HEK293 cells to fabricate the AAV and LV vectors (Schöll *et al.* 2013). A four-layer Cell Factory (CF4) system was used, which is the equivalent of approximately 15 cell culture flasks (175 cm^2). Contamination risk was kept low as the cell seeding, culture medium-changing and DNA transfection took place in a closed system with the emptying and refiling of media being conducted via a leveling bottle and connected tubing. Each layer was filled to 125 ml for cell seeding (for ca. 48hrs), 80 ml during transfection (ca. 8–14hrs) and 200 ml during virus production (ca. 60hrs). HEK293 cells were seeded to ca. 5 x 10^7 per Cell Factory layer.

HEK293 cells were shown to grow normally in the trays (figure 2) with growth kinetics similar to cells grown in conventional cell culture vessels; optimal density for transfection for example, was achieved after ca. 48 hours, and after a further 48 hours (following transfection) the cells (AAV) or supernatant (LV) were collected without issue for subsequent processing.



▲ Figure 1. A: Images showing similar cell density and morphology in the top, middle, and bottom layers of the Cell Factory system.
B: Images showing cell density in the entire growth surface of the top, middle, and bottom layers of the Cell Factory system. Note the growth pattern within the bottom layer due to vibrational effects during incubation; however, this is not detrimental to cell growth.

The Cell Factory system has also been used in the generation of recombinant viral vectors in order to investigate the mammalian neural circuits via optogenetic interrogation (Zhang *et al.* 2010). Here, the research team made use of low passage 293FT cells cultured in a CF4 system for the production of viruses. The culture and transfection process involved careful replacing of DNA-CaC_{l2} medium with a calcium phosphate-containing transfection mix, before subsequently replacing this transfection medium with fresh culture medium. The Cell Factory system greatly facilitated the aseptic exchange of multiple media types during the culture and transfection procedures. A protocol was eventually developed by the research team that may help to lend insight at the circuit level into the complex nature behind mammalian behaviors in health and disease.



▲ Figure 2. Green fluorescent cells beginning to produce recombinant AAV particles.

Expansion and differentiation of mesenchymal stromal cells

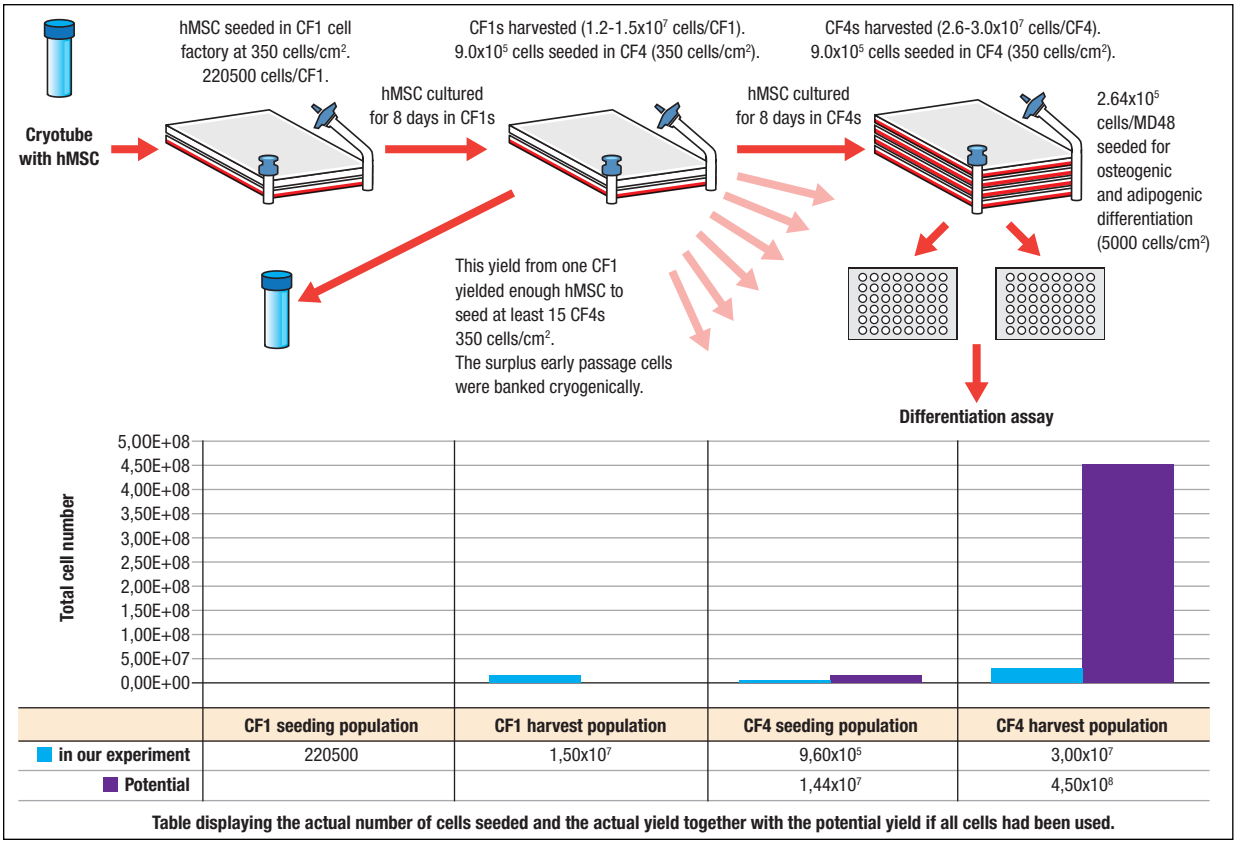
Human mesenchymal stromal cells (hMSCs) are excellent candidates for use in clinical research due to their immunomodulatory potential (English 2012) and ability to differentiate in to osteogenic (Gupta *et al.* 2011), chondrogenic (Xu *et al.* 2008) and adipogenic (Bork *et al.* 2011) lineages. hMSCs also have a great capacity for rapid expansion, something which can frequently be a limiting step in many investigations. A protocol has been developed to enable researchers to quickly expand populations of hMSCs, while maintaining their multipotency, making use of the Cell Factory system and Nunclon Delta surface (Carter *et al.* 2013).

Making use of either a mesenchymal stem cell basal or α-MEM growth medium, hMSCs were seeded at 350 cell/cm^2 and cultivated for eight days in a single layer Cell Factory (CF1) system. hMSCs were expanded on Nunclon Delta treated CF4s before being harvested and differentiated using either adipogenic or osteogenic differentiation medium supplemented with a growth supplement.

Cells showed normal growth and rapid expansion, with the yield from an initial CF1 being sufficient to seed at least fifteen CF4s (figure 3). In addition to large scale expansion, the hMSCs maintained their multipotency. This was verified following subsequent differentiation into osteoblasts or adipocytes using osteogenic or adipogenic differentiation media respectively.

The method developed by the researchers represents an excellent means of rapid, large scale expansion that can be applied to other cells types for use in a range of applications.

▼ Figure 3. Schematic of our in-house hMSC expansion protocol using Nunclon Delta treated Cell Factory systems.



	CF1 seeding population	CF1 harvest population	CF4 seeding population	CF4 harvest population
in our experiment	220500	1,50x10⁷	9,60x10⁵	3,00x10⁷
Potential			1,44x10⁷	4,50x10⁸

Table displaying the actual number of cells seeded and the actual yield together with the potential yield if all cells had been used.

Covering all cell culture bases

The Nunc Cell Factory system offers a highly effective, sterile solution for a wide range of cell culture needs, from small scale research to GMP scale operations.

Research shown here demonstrates that the Cell Factory system offers performance consistency from layer to layer – this is due to the Nunclon Delta surface: a fully synthetic, oxygen-enriched surface, increasing the hydrophilicity of what would otherwise be a hydrophobic polystyrene surface, promoting stable cell attachment.

The high level of inherent flexibility also allows the Cell Factory system to be adapted to a range of applications requiring aseptic cell culture and/or expansion. A plug and play system of connectors working within a closed system promotes efficient liquid handling and minimizes the risk of contamination. The Cell Factory systems also have a low footprint, with each CF10 using the same amount of space as 36 T175 flasks.

Thermo Fisher Scientific also provides a full line of equipment options, such as an automated Cell Factory manipulator (ACFM), CO_2 incubator and a shaker system, to support use of the Cell Factory system in an industrial scale.

The Cell Factory system is highly versatile and represents the ideal means of supporting the commercialization of research efforts from the bench to beyond.

For more information please visit:
www.thermoscientific.com/cellfactory

References

1. Bork, S. *et al.*, 2011. Adipogenic differentiation of human mesenchymal stromal cells is down-regulated by microRNA-369-5p and up-regulated by microRNA-371. Journal of Cellular Physiology, 226, pp.2226–2234.
2. Carter, S., Granchelli, J. & Stelzer, T., 2013. Large Scale Expansion and Differentiation of Human Mesenchymal Stromal Cells in the Thermo Scientific Nunc Cell Factory System. Thermo Scientific Application Note.
3. English, K., 2012. Mechanisms of mesenchymal stromal cell immunomodulation. Immunology and Cell Biology.
4. Gupta, D.M., Panetta, N.J. & Longaker, M.T., 2011. Osteogenic differentiation of human multipotent mesenchymal stromal cells. Methods Mol Biol, 698, pp.201–214.
5. Schöll, U., Zebski, M. & Kügler, S., 2013. Production of Viral Gene Transfer Vectors in Cell Factory Systems. Thermo Scientific Application Note.
6. Staggert, J., Lu, W. & Granchelli, J., 2013. An Examination of Cell Culture Performance on Multi-Layer Adherent Cell Culture Systems. Thermo Scientific Application Note.
7. Xu, J. *et al.*, 2008. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue engineering. Part A, 14, pp.667–680.
8. Zhang, F. *et al.*, 2010. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nature protocols, 5, pp.439–456.

www.thermoscientific.com/cellfactory

ANZ: Australia: 1300 735 292, New Zealand: 0800 933 966
Asia: China Toll-free: 800-810-5118 or 400-650-5118; India: +91 22 6716 2200, India Toll-free: 1 800 22 8374; Japan: +81 3 5826 1616; Other Asian countries: 65 68729717
Europe: Austria: +43 1 801 40 0; Belgium: +32 53 73 42 41; Denmark: +45 4631 2000; France: +33 2 2803 2180; Germany: +49 6184 90 6940, Germany Toll-free: 08001-536 376; Italy: +39 02 02 95059 or 434-254-375; Netherlands: +31 76 571 4440; Nordic/Baltic countries: +358 9 329 100; Russia/CIS: +7 (812) 703 42 15; Spain/Portugal: +34 93 223 09 18; Switzerland: +41 44 454 12 12; UK/Ireland: +44 870 609 9203
North America: USA/Canada +1 585 586 8800; USA Toll-free: 800 625 4327
South America: USA sales support: +1 585 586 8800
Countries not listed: +49 6184 90 6940 or +33 2 2803 2180

CSLSPCELLFACTY 0914

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand