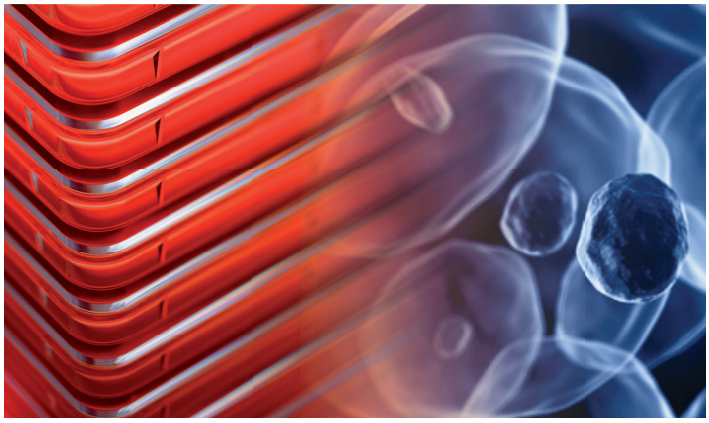
Optimized cell culture

For the 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 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 laboratory, 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 benchtop 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 multilayered solution designed for the cultivation of adherent cells ranging in scales: from a 1- or 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.

Consistency from top to bottom

Traditional multilayered 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.

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To address this concern, 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 (Cell Factory10): 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 Cell Factory10, 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 LV and AAV vectors (Schöll et al. 2013). A 4-layer Cell Factory (Cell Factory4) 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 changes, and DNA transfection took place in a closed system with the emptying and refilling of media being conducted via a leveling bottle and connected tubing. Each layer was filled to 125 mL for cell seeding (for about 48 hrs), 80 mL during transfection (about 8–14 hrs) and 200 mL during virus production (about 60 hrs).

HEK293 cells were seeded to about 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 about 48 hours; and after a further 48 hours (following transfection), the supernatant (LV) or cells (AAV) were collected without issue for subsequent processing.

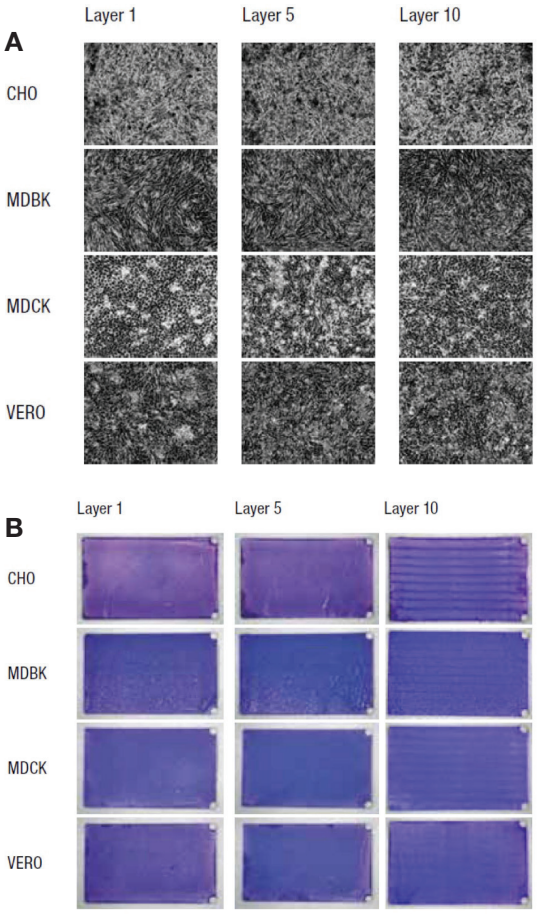


Figure 1. Consistency of growth between layers. (A) Images showing similar cell density and morphology in the top, middle, and bottom layers of the Cell Factory system. **(B)** Images showing cell density in the entire growth surface of the top, middle, and bottom layers of the Cell Factory system. Note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

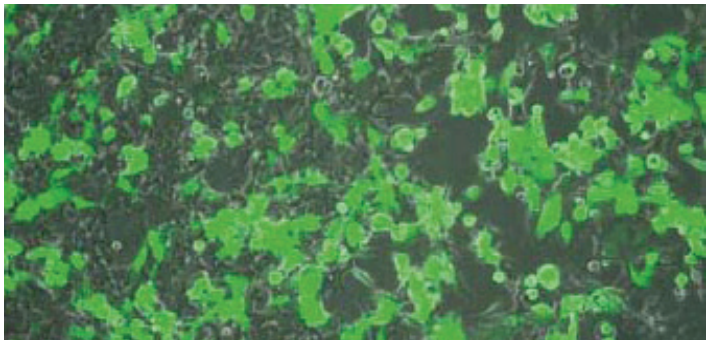


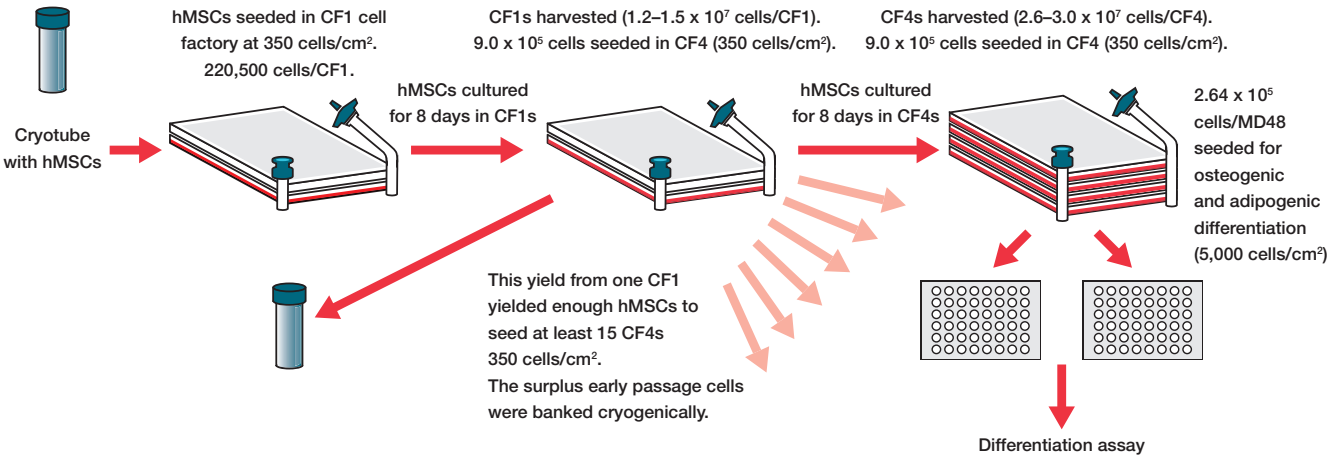
Figure 2. Green fluorescent cells beginning to produce recombinant AAV particles.

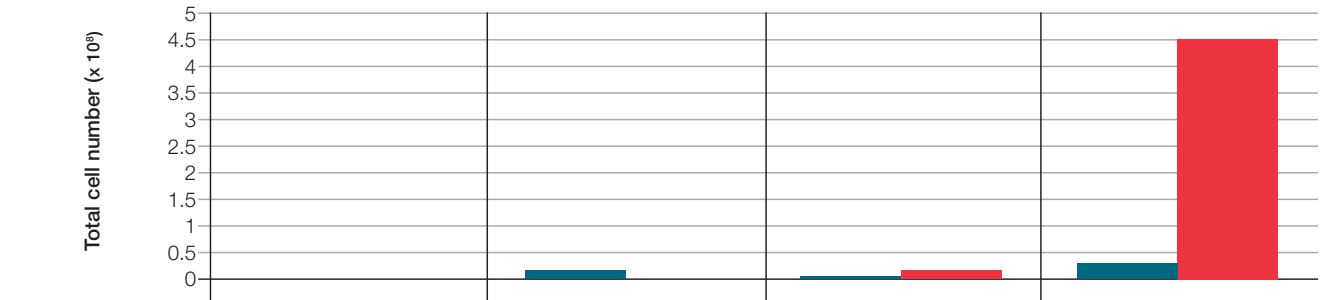
The Cell Factory system has also been used in the generation of recombinant viral vectors in order to investigate the mammalian neural circuits via optogenetic interrogation (Zhang et al. 2010). Here, the research team made use of low-passage 293FT cells cultured in a Cell Factory4 system for the production of viruses. The culture and transfection process involved careful replacing of DNA–$CaCl_2$ 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.

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 the 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 cells/cm^2 and cultivated for eight days in a single-layer Cell Factory (Cell Factory1) system. hMSCs were expanded on Nunclon Delta–treated Cell Factory4s before being harvested and differentiated using either adipogenic or osteogenic differentiation medium supplemented with a growth supplement.





	CF1 seeding population	CF1 harvest population	CF4 seeding population	CF4 harvest population
In our experiment	220,500	1.50 x 10^7	9.60 x 10^5	3.00 x 10^7
Potential			1.44 x 10^7	4.50 x 10^8

Figure 3. Schematic of our in-house hMSC expansion protocol using Nunclon Delta–treated Cell Factory systems. The table displays the actual number of cells seeded and the actual yield, together with the potential yield if all cells had been used.

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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 medium, respectively.

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

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, thereby 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 Cell Factory10 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, CO_2 incubator, and a shaker system, to support use of the Cell Factory system on 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.

References

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