APPLICATION NOTE

Master cell bank production of liver carcinoma cells

Improved productivity using a Nunc High Density Cell Factory 52-layer system

Introduction

Cells are the starting material of high-content screening, production of recombinant proteins, and cell-based assays. In order to ensure a steady supply of cells for these processes, cells are often banked in high numbers and resurrected when needed. This not only ensures that cells are available in sufficient numbers, but also reduces variability that might result from producing small batches of cells that tend to be unstable across generations.

Production of high numbers for cells requires a considerable investments in capital, energy, and planning. Many factors contribute to the unit productivity of cells, such as the liquid reagents, cell culture vessels, and of course the cells themselves. Even the best system can be limited by the constraints of the materials at hand. Productivity can often only be gained by adding more personnel, or buying more space.

Here, we report our initial experience with Thermo Scientific[™] Nunc[™] High Density Cell Factory[™] systems, which enable large-scale production of adherent cells. The multilayer design of the devices allowed us to use existing protocols and reagents (scaled for size) while switching from using six Cell Factory 10-layer systems (total of 60 layers) to three High Density Cell Factory 52-layer systems (total of 156 layers). Using these devices, we were able to significantly improve the total cell productivity (cells per batch per technician) of a liver carcinoma cell line without adding personnel or building space.

Materials and methods

Existing system: The existing production system consists of six Cell Factory 10-layer systems. The ergonomics of handling different devices of this size limits a technician to handling a maximum of six units per day. Because the productivity of these units is pooled at harvesting, the use of multiple units increases the likelihood that the entire batch might be contaminated. Due to space limitations, the two systems could not be compared head to head. Results are reported for typical experiments performed at different times.



Nunc High Density Cell Factory system: Three High Density Cell Factory 52-layer systems were connected in a closed tubing loop to reduce contamination (Figure 1). A filling and emptying tube assembly on one of the port openings of each system formed an aseptic connection between devices. The second port was fitted with a sterilizing air filter to ensure adequate gas diffusion within each system.

Cell culture: High Density Cell Factory 52-layer systems (and Cell Factory 40-layer systems) were seeded at a density of 1×10^5 cells/cm² (134.6 mL/layer, 7,000 mL total) using a proprietary RPMI-based medium that had been optimized for liver carcinoma cells. To improve adherence of this weakly adherent cell line, 1 µg/mL of poly-D-lysine was added to the medium. Cell Factory systems were transferred to a high-capacity incubator.

Harvesting cells: To facilitate handling of the Cell Factory systems, a cart of the same height as the incubator was used. This ensured that the systems could be easily transferred from the incubator and positioned on the laboratory bench for harvesting within a few minutes (Figure 2). The tubing length between the 52-layer systems was such that each system could be handled individually without compromising the connections. An additional tube was connected to facilitate harvesting from all three 52-layer systems within a safety cabinet. During harvesting, the medium was removed from each system individually. Adjacent systems were isolated by clamping the connecting tubing.

Each system was washed with 10 mL of PBS (without Ca²⁺ and MG²⁺) per layer PBS (without Ca²⁺ and Mg²⁺). The cells were harvested by emptying the 52-layer systems sequentially. To harvest the cells, 600 mL of GibcoTM StemProTM AccutaseTM Cell Dissociation Reagent was evenly distributed across the layers. To stop the dissociation, 400 mL of GibcoTM MEM-based inactivation medium was added. To collect any remaining cells, each system was washed again with 700 mL of medium per Cell Factory system by distributing the liquid across the surface of each layer. The individual harvests were transferred into 50 mL or 500 mL conical centrifuge tubes and then centrifuged at 180 x g for 10 minutes.

After centrifugation, the supernatant was aspirated with a pipette and each pellet was washed with 10 mL of Gibco[™] MEM-based cryomedium. The pellets were pooled to increase cell density. Each High Density Cell



Figure 1. Configuration of High Density Cell Factory 52-layer systems.



Figure 2. Setup for harvesting from High Density Cell Factory 52-layer systems.

Factory 52-layer system yielded approximately 20 mL of cell suspension. Cell suspension samples were stained and counted using trypan blue, and cell viability was assessed using a standard hemocytometer. The harvest was adjusted to a final total volume of 130 mL in Gibco MEM- based cryomedium, providing a final count of 1.0 x 10⁸ cells per 2.0 mL cryovial. The cryomedium was premixed with 6 mL of DMSO, for controlled freezing of the cells and to mitigate ice crystal formation in the cell plasma membranes. The cells were resuspended to a final volume that resulted in a DMSO concentration of 5% in the cryomedium.

Sixty-five Thermo Scientific[™] Nunc[™] Non-Coded Cryobank Vials* (Cat. No. 374223) were filled using an automated protocol (Sartorius[™] Fill-it), then transferred to a controlledrate freezer where the sample temperature was reduced to -80°C. The cells were stored at -80°C overnight before transfer to storage in liquid nitrogen (-180°C) for long-term storage.

* For Invitro Diagnostic Purposes.

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Results

Yields were calculated from separate culture trials and compared to total yields achieved using different Cell Factory system configurations (Table 1). Trials reflect separate experiments using these configurations and were not done contemporaneously.

Table 1. Yields from different Cell Factory configurations.

System configuration*	Total yield
Six Cell Factory 10-layer systems	2.08 x 10 ⁹ cells
Three Cell Factory 40-layer systems	4.80 x 10 ⁹ cells
Three High Density Cell Factory 52-layer systems	6.71 x 10 ⁹ cells

Yields expressed are nominal, and typical variation of $\pm 15\%$ between harvests is routinely observed.

Discussion and conclusions

In the current example, we were able to produce a significantly greater (~3x) total cell yield in the High Density Cell Factory 52-layer system, while making improvements to the ergonomics and efficiency of harvesting such that the total yield of cells per batch was greater. Even more impressive was that the yield per unit of surface area was also greater (54,842/cm², 63,291/cm², and 68,058/cm², respectively, for 10-, 40-, and 52-layer devices).

The important features of this system that lend itself to adoption were that the device itself is a minor modification to the existing design of the Cell Factory system (same materials and surface treatment). These common design elements allowed cells that had been adapted to a Cell Factory system to adapt in a single passage to growth on a High Density Cell Factory 52-layer system. Larger batch sizes were accomplished using a closed-loop system enabled by the same port designs and same collection of connectors and tubing sets. The closed system reduced the chance of contamination from handling multiple smaller devices. The larger batch sizes also had downstream benefits in terms of a reduction in the individual quality control checks needed on smaller batches.

Current cell production is constrained by personnel and space. The amount of space in a hood limits the number of Cell Factory systems that can be handled in each configuration by a single technician (six 10-layer, three 40-layer, or three 52-layer systems). Adding more hood space or more personnel adds significant costs, so there is a powerful incentive to find ways to improve total yield with existing resources. The High Density Cell Factory 52-layer system is a tool that allows for creative improvements to the workflow with minimal changes to the underlying process.

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