Introduction

New Brunswick Scientific now offers a new labor-saving device for producing mg quantities of proteins, virus or cell mass from anchorage-dependent or suspension cultures. More productive than traditional roller bottles, spinners or T-flasks\(^1\), the new FibraStage\(^\circledast\) system is inexpensive, simple to operate, and disposable. Typical yields of \(4.9 \times 10^9\) cells per 500 mL disposable FibraStage bottle have been achieved with a variety of cell types. The system holds up to four bottles, enabling simultaneous screening of four different cell lines or media compositions.

About the FibraStage\(^\circledast\) Single-Use Cell Culture System

At the heart of the FibraStage system are pre-sterilized, single-use bottles, with a cell growth chamber filled with 10 grams of FibraCel\(^\circledast\) disks. FibraCel is a three-dimensional growth substrate, composed of polypropylene and polyester non-woven fiber, which provides an extremely high-surface area for cell growth. Anchorage-dependent and suspension cultures become attached to or embedded in the FibraCel disk bed, where they remain throughout the growth process. Immobilization within the disk bed not only protects the cells from damage by shear forces, but also simplifies harvesting, allowing the supernatant to be easily recovered from the cell-free medium. The bottles come pre-sterilized and ready to use, requiring no cleaning, autoclaving or validating, for further reduction of labor.

The FibraStage system includes a variable-speed platform into which the FibraStage bottles are placed. When the platform is raised, bellows at the base of each bottle are compressed, forcing media over the cells, facilitating nutrient uptake and removal of cell waste. When the platform is lowered, the bellows expand, allowing the media to recede, leaving the disks covered by a thin liquid film. The film helps with oxygen transfer and removal of excess CO\(_2\). The entire device fits into a standard CO\(_2\) incubator for control of temperature and gasses, and the controller is magnetized for convenient storage on the outside wall of the incubator.

Fig. 1. The FibraStage system accommodates up to four disposable 500 mL bottles, pre-filled with FibraCel disks for high-yield growth of anchorage-dependent or suspension cultures.

FibraCel disks have a proven record of high-yield production of cells, virus and secreted proteins from a wide variety of cell lines, including hybridoma, CHO, VERO, BHK, and insect cells\(^2\). These disks are also currently being used in the production of the world’s first licensed gene therapy drug, a treatment for head and neck squamous cell carcinoma. New Brunswick Scientific offers a wide range of bioreactor systems incorporating the FibraCel packed-bed technology in 0.5 to 150 Liter working volume capacities, but FibraStage is the first of their single-use systems for cell culture.

The Effect of Media on Cell Attachment

We have found that approximately 85% of suspension cells and 100% of anchorage-dependent cells we’ve tested will readily attach to FibraCel disks. If in doubt, the most economical way to determine if your cells will attach to the disks is to experiment in T-flasks. In the US and parts of Europe, NBS also offers FREE 30-day in-lab trials of the FibraStage system. Ask your NBS representative for details.
Once you’ve verified that your cell line will grow using FibraCel disks, inoculate one to four FibraStage bottles, as desired. Begin with an inoculum of at least $1 - 2 \times 10^8$ cells per bottle for mammalian cell cultures, and $1.5 - 2 \times 10^8$ cells per bottle for insect cell cultures. (See Table 2.) Higher cell densities will shorten the lag phase of the culture.

Open the bottle cap and add 500 mL of media including the inoculum. Place the bottle(s) in the FibraStage device, and place the entire system inside a standard CO2 incubator. Set temperature to 37°C for mammalian cells and 28°C for insect cells.

### Table 1. Serum-Free Media Recommendations by Cell Line

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Serum Free Media</th>
</tr>
</thead>
</table>
| CHO       | CHO-S-SFM II (Gibco)  
CHO III A (Gibco)  
CHO-A-SFM (Gibco)  
ExCELL 301 (JRH)  
HyQ PF-CHO (Hyclone)  
HyQ-CCM 5 (Hyclone)  
HyQ SFX-CHO (Hyclone)  
IS-CHO-CD (IRVINE), proCHO4cdm |
| HEK-293   | EX-CELL 293 (JRH), Pro293a-CDM (CAMBREX)  
HyQ SFM4 HEK293 (Hyclone) |
| VERO      | VP-SFM (Gibco)  
ICN-VERO (ICN), PEEK-1 (Biochrom) |
| Sf-9      | SF-900 II (Gibco)  
EX-CELL 420 (JRH)  
EX-CELL 400 |
| Hi-5      | EXCEL405(JRH), Express (Gibco) |
| Hybridoma/NSO | EX-CELL 610-HSF (JRH) |

### Table 2. Inoculum Density

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Inoculation Density (cells per bottle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO, VERO</td>
<td>$1 - 2 \times 10^8$</td>
</tr>
<tr>
<td>HEK-293, Sf-9, Hi-5</td>
<td>$1.5 - 2 \times 10^8$</td>
</tr>
</tbody>
</table>

Adjust the FibraStage controls as per Table 3. **Up (Rise)** and **Down Rate** will adjust the speed of the platform, which creates gentle mixing of media to ensure cells have sufficient nutrients and oxygen. Increasing **Top Hold Time** allows nutrient exchange between media and disks and increases CO2 level in the culture. Increasing **Bottom Hold Time** will increase oxygen level in the culture, allowing sufficient time for oxygen transfer from air to cells and helps stabilize the pH.

It is important to note that increasing the Up Rate facilitates mixing the culture medium, but also increases shear stress to cells. Increasing the Bottom Hold time facilitates aeration efficiency, but also reduces the mixing frequency of culture medium. Longer Bottom-Hold times tend to promote initial cell propagation due to the concentration of cells, however, longer Bottom-Hold times may also restrict nutrients available to the cells.

### Table 3. Control Settings — Inoculation

<table>
<thead>
<tr>
<th>Up (Rise) Rate</th>
<th>Top Hold Time</th>
<th>Down Rate</th>
<th>Bottom Hold Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 mm/sec</td>
<td>20 sec</td>
<td>2.0 mm/sec</td>
<td>0 sec</td>
</tr>
</tbody>
</table>

### Culture Phase

After 2 - 3 hours of inoculation, reset the control parameters and Up/Down Hold Time as indicated in Table 4.

We have found that 90% of the tested cell lines will become immobilized in the disks within approximately 30 – 45 minutes. During the run, monitor the substrate and metabolite concentrations including glucose, glutamine, ammonia and lactate once a day. Media and glucose should be added as required.
**pH Measurements & CO₂ Concentrations:**

Due to the high cell densities typically achieved in FibraCel culture, the pH levels in the medium may fluctuate in the later stages of cell growth.

Adjust the culture medium pH to a range of between 7.2 to 7.4 for mammalian cell culture, 6.2 to 6.4 for Sf-9 insect cells, and 6.4 to 6.5 for Hi-5 cells.

When growing mammalian cell cultures, a pH level above 7.6 may slow attachment of your cells to the disks. If the culture medium contains 2.2 g/L NaHCO₃, set the CO₂ to 5%. If pH is below normal range, adjust the CO₂ concentration to 0, and/or increase the NaHCO₃ concentration to 3.7 g/L. Culture medium containing 25 mM HEPES can also stabilize the pH during culture.

In insect cultures, when the pH is below 6.0, add 1M of Bis-Tris to increase the pH to 6.5. Increasing the Up/Down Speed and Bottom Hold Time will help to expel CO₂, reduce lactate production, and thus further stabilize pH in culture medium.

Measure the pH regularly, and adjust the CO₂ concentration accordingly in the incubator.

---

**Media Consumption:**

The medium consumption is process dependent. Expect to use 1 to 3 L of media for virus production in semi-batch culture. In processes where you are continuously collecting conditioned culture medium for secreted protein production, you may use 5 to 10 L of media to extend the culture to about 15 days.

**Media Replenishment**

Replenish or modify the culture media as necessary:

- **Glucose Concentration** - Change the medium when the glucose concentration falls below 1.0 – 1.5 g/L (initial glucose concentration should be > 3.5 g/L).

- **pH** - Adjust the pH when the pH falls below normal range; glucose concentration should remain above 1.0 - 1.5 g/L.

- **CO₂** – If the CO₂ concentration at incubation has been set to 0, add buffer or change the medium. For cultures that extend over weeks or months, exchange the medium once a day. Replace the medium with 500 mL each day to keep cells in an optimal state. If the media is rich enough and does not require frequent replenishment, monitor the pH and adjust the CO₂ concentration to NaHCO₃, 1 M HEPES. If working with insect cells, adjust with 1 M Bis-Tris.

---

**Table 4. Control Settings — Culture Phase**

<table>
<thead>
<tr>
<th>Cell Type / End Product</th>
<th>Up (Rise) Rate</th>
<th>Top Hold Time</th>
<th>Down Rate</th>
<th>Bottom Hold Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybridoma / Secreted &amp; Non-Secreted Products</td>
<td>2.0 mm/sec</td>
<td>20 sec</td>
<td>2.0 mm/sec</td>
<td>1 - 90 min</td>
</tr>
<tr>
<td>All Other Cell Lines with Growth-Associated Virus / Cell Mass Production – Secreted &amp; Non-Secreted Products</td>
<td>1.0 - 1.5 mm/sec</td>
<td>10 sec</td>
<td>1.0 - 1.5 mm/sec</td>
<td>10 sec</td>
</tr>
<tr>
<td>All Other Cell Lines with Non-Growth -Associated Productivity (e.g. slow growth condition productivity) / Secreted &amp; Non-Secreted Products</td>
<td>1.0 mm/sec</td>
<td>10 sec</td>
<td>1.0 mm/sec</td>
<td>30 - 90 min</td>
</tr>
</tbody>
</table>

*Rate and hold time ranges will vary depending on the specific cell line and medium*
Estimating Number of Cells:

You may estimate the viable cell count during culture in either of three ways. The cell densities should be periodically measured:

- **Nucleus Count** - Each FibraStage system includes 500 mL of crystal violet dye (CVD) for estimating the number of cells using the nucleus count method. Aseptically remove a few samples of the FibraCel disks using the sterilized long-arm forceps provided, and estimate the cell population by crystal violet dye (CVD) nucleus count method, which is further described in our FibraStage operating manual. Each FibraStage bottle contains 10 grams of disks, equaling 2,210 disks, ±1%.

- **Glucose Uptake Rate (GUR)** - Estimate the cell growth by calculating the GUR, which should be proportional to the total cell volume. We recommend you measure glucose concentration every day or approximately 6 to 8 hours after medium replenishment. Use this equation:

  \[ \text{GUR} = \left( C_{\text{Glu}, t_2} - C_{\text{Glu}, t_1} \right) \times V_{\text{solu}} / (t_2 - t_1) \]

  Where GUR is the Glucose Uptake Rate in mg/hour
  \( C_{\text{Glu}} \) is the concentration of residual glucose
  \( t_1 \) & \( t_2 \) are the sampling hours
  \( V_{\text{solu}} \) is the volume of media in liters

  We recommend maintaining the GUR at > 1,000 mg/L.

- **NucleoCounter Automated Cell Counter** - Or, use an automated cell counting device, like the NucleoCounter®, available in the US exclusively through New Brunswick Scientific. The NucleoCounter is an automated cell counting device specifically designed to provide fast and accurate counts of mammalian and insect cells, without regard to cell size or morphology. (A NucleoCounter YC-100 model is also available for automated counts of yeast cells.) The NucleoCounter systems rely on use of a disposable plastic sampling cartridge — the NucleoCassette®, which contains a nucleus staining dye, propidium iodide. To use, aseptically remove a few samples of the FibraCel disks and remove the cells from the disks. How cell removal is accomplished is dependent on whether you are culturing suspension or anchorage-dependent cells:

  **Suspension cells** are usually released from the FibraCel disks very easily. Place the disks in a small test tube containing 5 mL of phosphate-buffered saline (PBS) and shake, sonicate or vortex. The cells should fall off the disks. Remove the PBS and centrifuge the liquid; re-suspend the cells in 0.5 mL PBS.

  **Anchorage-dependent cells** may require sonication or trypsinization to remove the cells from the FibraCel disks. For trypsinization, place the disks in a test tube. Using a standard trypsinization buffer, incubate for 30 seconds, remove the liquid, add 1 mL PBS and incubate at 37°C for 8 minutes. This suspension can then be treated for use in the NucleoCassettes.

Once you have removed the cells from the disks, immerse the tip of the NucleoCassette into your sample, which aspirates a predefined volume of your sample into the cassette. The sample dissolves the propidium iodide dye, which has been immobilized inside the cassette. The cassette is then placed into the reading chamber of the NucleoCounter, activating the measurement cycle. A mechanical drive initiates the flow of sample into the counting chamber of the NucleoCassette, an image of the nuclei is recorded and analysis of the image occurs. In seconds, the count will be shown on the front panel display screen of the NucleoCounter. An optional, proprietary software program allows you to capture both an image and a numeric count for data logging purposes.

Harvesting:

You can harvest cells, cell components, or intracellular protein by trypsinization, lysis buffer, freeze-and-thaw, sonication or any chemical or physical method. Specific protocols are available in New Brunswick Scientific’s FibraStage Operating Manual.

Results and Summary:

Table 5, shown on the next page, provides data from a few of our recent studies using HEK-293, Sf-9 and CHO cells. These results were achieved using a single FibraStage bottle. For details of specific protocols, contact NBS. All results vary, and depend on the cell line, medium, nutrients and control parameters.
Table 5: Summary of Results Using Various Cell Lines

<table>
<thead>
<tr>
<th>Description</th>
<th>HEK-293</th>
<th>Sf-9 Insect Cells</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating Mode</td>
<td>Semi-Batch</td>
<td>Batch</td>
<td>Semi-Batch</td>
</tr>
<tr>
<td>Medium</td>
<td>MEM / 10% FBS</td>
<td>SF 900 II</td>
<td>DMEM/5% FBS, 2.5 g Glucose, 3.7 g NAHCO3</td>
</tr>
<tr>
<td>Inoculation Cell Density</td>
<td>2.5 x 10^5</td>
<td>1.5 x 10^8</td>
<td>5 x 10^7</td>
</tr>
<tr>
<td>Maximum Cell Density</td>
<td>2.8 x 10^6</td>
<td>1.4 x 10^7</td>
<td>1.4 x 10^9</td>
</tr>
<tr>
<td>Protein Production</td>
<td>3.7 pmol mg^-1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Virus Production</td>
<td>—</td>
<td>3.45 x 10^{12} (TCID_{50})</td>
<td>—</td>
</tr>
</tbody>
</table>

Conclusion:

FibraStage System is a novel disposable bioreactor that is easy to use, is space-saving and labor-saving and achieves high cell densities compared to other 2-D bioreactors.

For further information or to request quote, please send an e-mail to bioinfo@nbsc.com or see New Brunswick Scientific’s web site at: www.nbsc.com/btb.htm

References:
