Whitepaper

Producing MSC-derived Cell Therapies from Isolation to Large-scale Expansion; a Journey of Workflows, Technologies, and Case Studies

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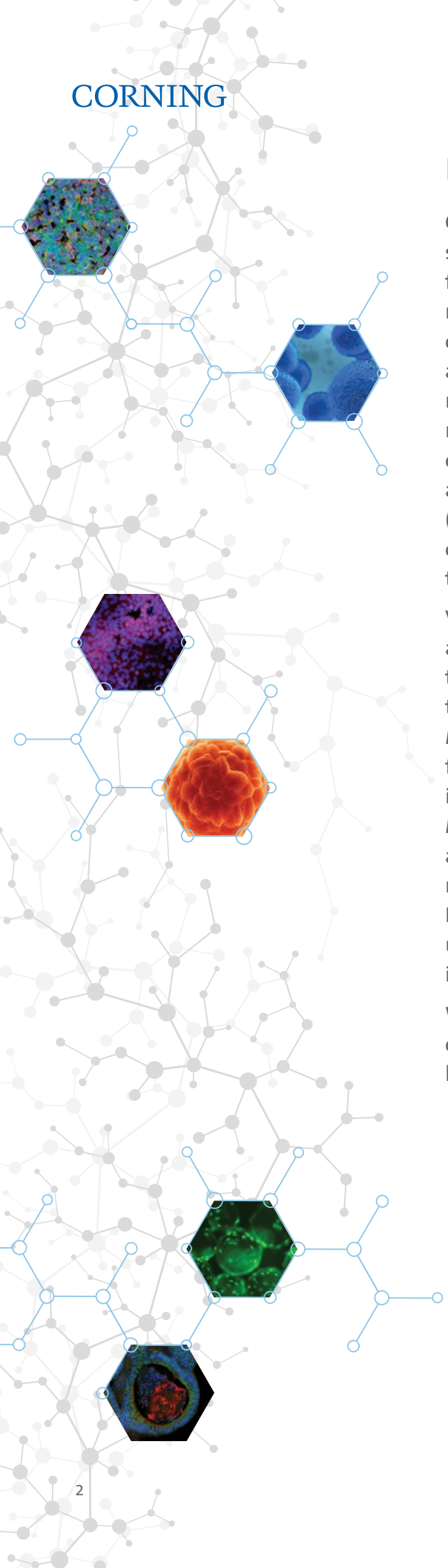


Introduction

Over the past decade, stem cell research has made significant strides and has increasingly been applied as a therapeutic method for various disorders. Stem cells, by definition, are specialized cells naturally found in most body tissues, capable of self-renewal and differentiation into diverse cell types. Essentially, stem cells serve as the body's natural toolkit for maintaining tissue integrity and repairing injuries. With their abilities to promote tissue healing, replace lost or damaged cells, and function as biofactories and delivery platforms, stem cells hold the potential to target a wide array of diseases. Both autologous (self-derived) and allogeneic (donor-derived) approaches are being explored, with allogeneic options being particularly appealing due to their potential "off-the-shelf" readiness and clinical availability.

Various types of stem cells are under investigation for therapeutic applications, and their progress towards clinical use varies. Among these, mesenchymal stem/stromal cells (MSCs) have been leading the way, outpacing other multipotent and pluripotent cell types. MSCs boast diverse biological functionality that can be harnessed for therapeutic purposes. These multipotent cells can differentiate into osteoblasts, adipocytes, and chondrocytes. More importantly, MSCs possess immunomodulatory and anti-inflammatory properties, acting as the body's mobile paramedics. This innate functionality makes them powerful agents for tissue regeneration and repair. Furthermore, MSCs are immune-evasive, allowing them to be used as allogeneic therapies without the need for patients to be immunosuppressed.

With their unique capabilities and therapeutic potential, MSCs continue to be at the forefront of regenerative medicine, offering hope for treating a broad spectrum of diseases.

Chapter 1: MSCs as Biological Therapeutics

Mesenchymal stem/stromal cells are incredibly responsive to inflammation. These highly motile cells migrate to areas of tissue damage to assist with repair. They secrete a variety of cytokines, chemokines, and growth factors that help regulate inflammation, angiogenesis, and fibrogenesis. One of the remarkable features of MSCs is their ability to produce higher concentrations of extracellular vesicles (including exosomes) than other cell types, making them valuable *in vivo* biofactories for delivering molecules of interest. Moreover, MSCs can be engineered to overexpress specific proteins, growth factors, or siRNA, allowing for precise targeting when injected directly at the site of interest. They can be harvested from various tissue sources, making them readily available for therapeutic purposes.

Given their complex *in vivo* microenvironment, manufacturing MSCs requires thorough consideration to maintain the relevant conditions associated with the therapeutic product. While MSCs can be expanded in adherent-based culture systems, most sources have limited population doubling before reaching senescence. Despite these challenges, MSCs remain a compelling option for both allogeneic and autologous cell therapies across a wide array of diseases.

In this review, we share insights into the development of production processes for MSC-based therapies. Drawing from the experiences of a global team of Corning Application Scientists with diverse backgrounds in cell and gene therapy, we offer our learnings from working with academic and translational researchers, as well as biotech and pharmaceutical companies pushing the boundaries of MSC-based drug development.

Chapter 2: Target Disease Indications and Applications

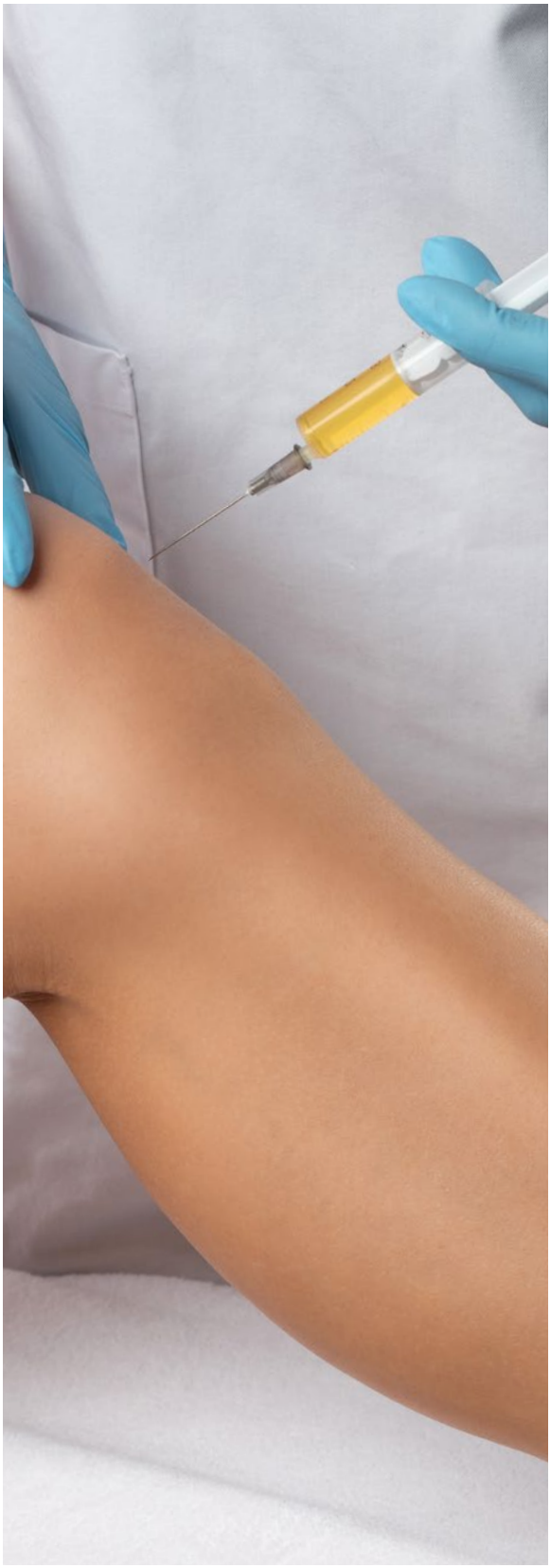
Stem cell therapy has traditionally been applied to hematopoietic disorders through bone marrow transplants. Recently, though, stem cell-derived therapies have been pursued for treating a wide range of non-hematologic disorders. Currently, MSC-based clinical trials are being conducted for various pathological conditions, with many completed trials demonstrating both safety and efficacy. Initially, MSCs were used to improve organ transplantation outcomes by targeting graft-versus-host disease (GVHD) due to their immunomodulatory properties. Now, multiple clinical trials are focusing on diseases affecting various organ systems, including the liver, kidneys, heart, bones, cartilage, nervous system, and skin (wound healing).[1]

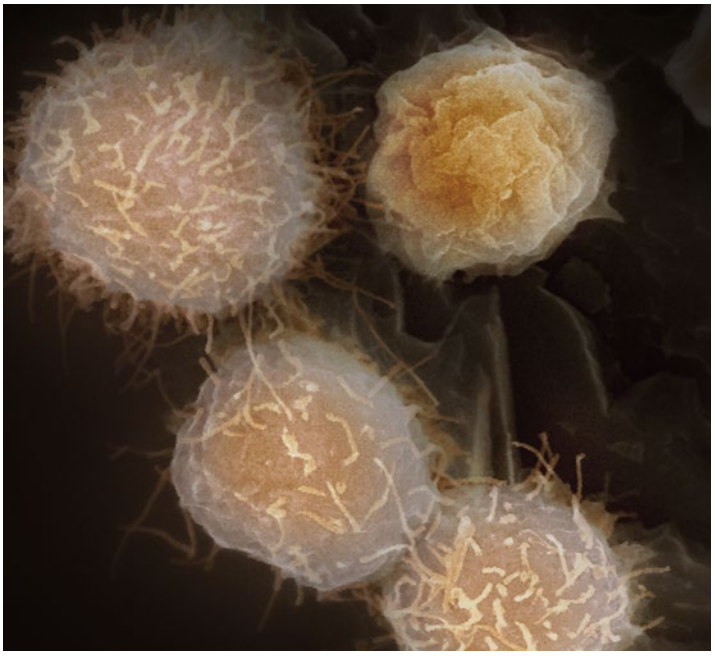
The immunomodulatory and anti-inflammatory features of MSCs have encouraged researchers and clinicians to target diseases believed to have an inflammatory etiology. While not exhaustive, a select number of disease targets for MSC therapy are summarized (Table 1)[2], detailing the infusion methods (intrathecal (I.T.) or intravenous (I.V.)), required cell numbers, MSC sources (bone marrow, Wharton's jelly, or umbilical cord), and clinical trial phases. Here are some examples of how MSCs are being applied:

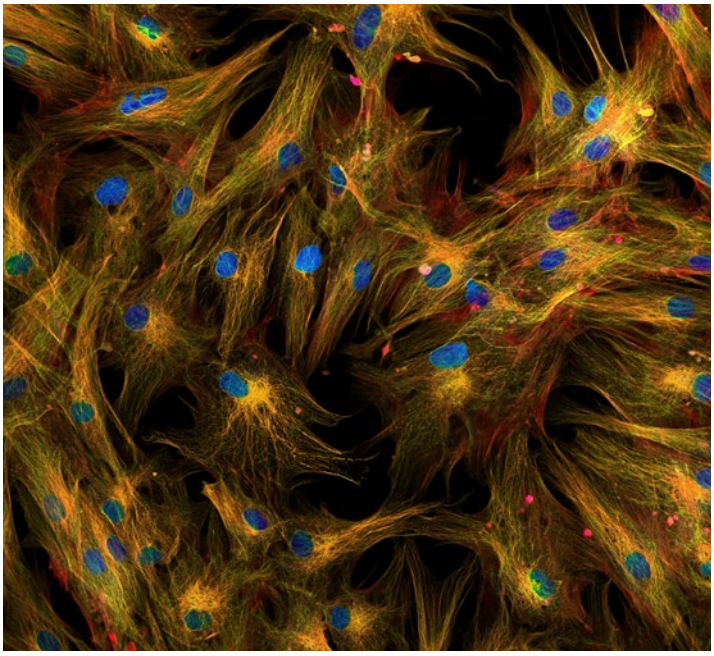
- **Kidney Disease:** Inflammation significantly contributes to renal cell damage, potentially leading to chronic kidney disease. MSC-derived cell therapy has shown to improve remission of kidney disease by enhancing the estimated glomerular filtration rate (eGFR) and lowering blood creatinine levels after one year of treatment.[3]
- **Liver Diseases:** MSCs have the potential to differentiate into hepatocyte-like cells, making them suitable for treating liver diseases. Bone marrow- and umbilical cord-derived MSCs have shown therapeutic responses in liver cirrhosis and ischemic liver injury.[4]
- **Cartilage Tissue Engineering:** The ease of isolating and expanding MSCs, along with their multipotential differentiation capacity, particularly chondrogenic differentiation, makes them ideal for articular cartilage tissue engineering. This aims to replace and regenerate the diseased structure in joint diseases like osteoarthritis (OA) and rheumatoid arthritis (RA).[5]
- **Neurological Disorders:** Many neuromuscular and neurodegenerative conditions, such as Amyotrophic Lateral Sclerosis (ALS) and some autism syndromes, are thought to be associated with inflammation. These conditions are potential targets for MSC-derived therapies due to MSCs' immunomodulatory and anti-inflammatory properties.[6]

Table 1. Summarized disease targets for MSC therapy.

Disease	Infusion Method	Cell Number	MSC Source	Clinical Phase
ALS	Intrathecal	1.5×10^7	Bone Marrow	I/IIa
Parkinson's Disease	Intrathecal	2.5×10^6/kg	Bone Marrow	II
Spinal Cord Injury	Intrathecal	1.0×10^7	Wharton's Jelly	I/IIa
Stroke	Intravenous	1.0×10^6	Bone Marrow	II
Primary Biliary Cirrhosis	Intravenous	0.5×10^6/kg	Umbilical Cord	N/A
Chronic Kidney Disease	Intravenous	$1\text{-}2 \times 10^6$/kg	Bone Marrow	I







Chapter 3: Sources for MSCs

MSCs are a broad term used to describe "stromal" cells derived from various tissue sources. The exact definition of an MSC has historically been a source of debate. In 2006, the International Society for Cellular Therapy (ISCT) established minimal criteria for identifying MSCs. According to ISCT, MSCs must:[7]

- Be multipotent
- Have tri-lineage differentiation potential into osteogenic, adipogenic, and chondrogenic lineages
- Positively express key cell-surface markers CD90, CD105, and CD73
- Lack expression of cell surface markers CD45, CD34, CD14, CD79, and HLA-DR
- Be capable of adhering to tissue culture flasks

Cells meeting these criteria can be isolated from various tissue sources, with the most common being bone marrow, umbilical cord (Wharton's jelly), placenta, adipose tissue, and adult dental pulp. Additionally, MSCs have been isolated from other biological niches, such as the endometrium, human exfoliated deciduous teeth (baby teeth), and breast milk.

When selecting a source material for MSCs, it is essential to consider the availability, therapeutic characteristics, and manufacturability of the cells derived from each source. Here are some key points to keep in mind:

- **Bone Marrow and Adipose Tissue:** Harvesting MSCs from these sources can be invasive and may require a biopsy. However, these sources are well-characterized and widely used.
- **Birth Tissues (Umbilical Cord and Placenta):** These sources offer a non-invasive option but suffer from limitations in the availability of material.
- **Baby Teeth and Breast Milk:** These methods are non-invasive and readily available, but they are less established, and the cells are not as well characterized.

MSCs derived from different sources often retain characteristics associated with their tissue of origin. For example, bone marrow-derived MSCs more readily differentiate into bone cells, while adipose-derived MSCs are more prone to differentiate into fat cells. The age and health status of the donor can also affect the quality of the cells. Younger donors typically provide cells with higher proliferation and differentiation potential.

Selecting the appropriate tissue source for MSCs can significantly impact clinical outcomes. For instance, a clinical trial highlighted that while bone marrow and Wharton's jelly-derived MSCs had positive effects on symptoms, adipose tissue-derived MSCs did not, emphasizing the importance of tissue source selection on clinical outcomes.

Chapter 4: Common Methods for MSC Isolation

Various protocols have been established to mitigate the risks associated with source variability and to limit the generation of impure cell populations. Initially, isolation methods from bone marrow or adipose tissue used tissue culture plastic to select for the adherent cell population. This method results in a cell population primarily composed of MSCs with minimal contamination from other cell types.

Isolation methods have since improved and can be categorized into 2 main procedures: enzymatic methods and explant cell culture techniques.

Enzymatic Methods

- Involves using up to 3 proteolytic enzymes such as collagenase, dispase, or trypsin to digest the tissue.
- The resulting cell mixture is then plated on tissue culture plastic in a relevant cell culture medium.
- Cells established via enzymatic methods typically reach confluence in about 7 days.
- A potential downside is that over-digestion can damage cells, and growth factors like bFGF and PDGF may be needed to establish primary cell culture. Despite this, enzymatic methods are often preferred for their higher cell yields and shorter timeframes, making them suitable for clinical applications requiring speed.

Explant Cell Culture Techniques

- Do not use enzymes to establish primary cell culture.
- Tissue is plated, and cells migrate out from the tissue and adhere to the plastic surface.
- Can take up to 15 days for cells to fully populate a plate and reach confluence.
- Explant methods are favored for generating cell banks with lower population doubling times (PDTs), minimal impact on surface marker expression, and no need for additional growth factors, reducing overall cell isolation costs.

Enhanced Tissue Culture (TC)-treated plastic, such as the Corning® CellBIND® surface, can improve overall isolation yields for both enzymatic and explant techniques.

An optimized isolation process ensures high-quality cells feed into MSC manufacturing. At the time of isolation, it's crucial to consider generating relevant cell banks to avoid genetic or biological drift during cell therapy development and manufacturing. Creating both a working cell bank (for process development) and a master cell bank (for manufacturing) helps maintain cell performance. For MSCs, it's generally recommended not to extend beyond 15 population doublings, which should be considered when determining the size of each cell bank.

Comparing MSC Isolation Methods

Isolation Method	Time to Confluence	Initial Cell Yield	Cell Health and Viability	Population Doubling Time	Impact to Surface Marker Expression	Growth Factor Addition to Isolation Medium	Cost of Cell Isolation
Explant	2 weeks	Lower	Higher	Shorter	Lower	No	Lower
Enzymatic	1 week	Higher	Lower	Longer	Higher	Yes	Higher

Chapter 5: Media Options and Considerations for Production of MSCs

Cell culture media typically consist of a basal formulation (containing sugars, vamino acids, and salts) and a supplement (containing bio-active factors such as growth factors, cytokines, proteins, and extra-cellular vesicles). In recent years, there have been extensive efforts to develop media specifically for MSCs. It is crucial to consider the combination of the basal media formulation and its supplements. Often, if the supplement is changed, the basal media formulation needs to be adjusted to compensate. As the primary "food" for cells, the media formulation is key to maintaining desired cell quality attributes. Since media can directly impact cell biology, it is critical to test media performance based on established critical quality attributes.

Traditionally, fetal bovine serum (FBS) paired with either DMEM or Alpha-MEM basal media has been the go-to complete media formulation for MSCs. FBS is a complex, animal-derived supplement containing proteins, growth factors, and cytokines essential for cell adhesion, proliferation, and gene expression maintenance. While FBS offers a rich mix of nutrients and biological factors that support cell growth, it poses challenges due to lot-to-lot variability and potential safety concerns, such as immune reactions or transmission of adventitious agents. Although FBS can still be used in cell therapy manufacturing, care must be taken to ensure all components are thoroughly removed from the final product.

To address these concerns, serum-free (SF) formulations have been developed for many cell lines, replacing serum with other animal-sourced proteins. However, these still suffer from variability and potential safety issues associated with animal components.

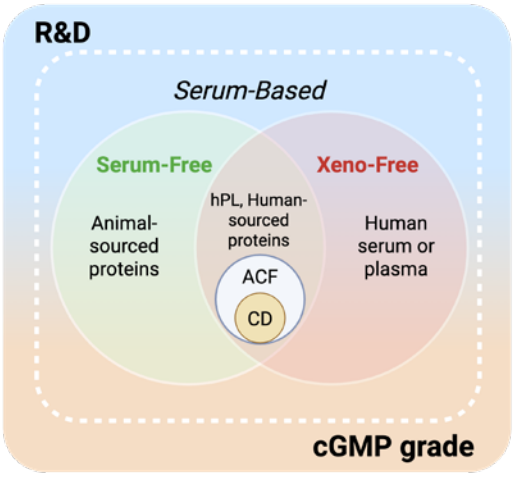
As an alternative, many production workflows are moving towards human-derived supplements like human platelet lysate (hPL).

These xeno-free (XF) formulations reduce safety concerns and decrease lot-to-lot variability due to hPL being less complex than FBS. Eliminating non-human materials makes these formulations more suitable for clinical applications by reducing immune response risks.

For even greater risk reduction, animal component-free (ACF) media formulations exclude any animal-derived components, including human-sourced materials. These provide a higher level of safety for biopharmaceutical applications compared to XF formulations.

An ideal media formulation for cell therapy manufacturing would be fully defined. Chemically defined (CD) and synthetic components to support cell growth. However, developing CD media can be costly and requires an in-depth understanding of cellular requirements. Currently, there are no high-performing CD media for MSCs known to the authors. For therapeutic applications, it is also essential to ensure that the media are manufactured under cGMP conditions and come with the necessary documentation for production use.

Several specialty media formulations have been specifically developed to support MSC growth, expansion, and maintenance of multipotency without the concerns associated with FBS. Transitioning to non-serum-based media requires careful optimization. MSCs are sensitive to changes in their microenvironment, and the absence of serum can alter their proliferation rates, morphology, marker expression, or differentiation ability. Additionally, the cost of these specialty media remains a key consideration. While SF, XF, and ACF media offer consistency and are preferred for clinical-grade manufacturing, their higher price compared to serum-containing media may limit their use in large-scale production.



Media Type	Key Features	Cell Types
Thermo Fisher StemPro™ MSC SFM	Xeno-free	hMSC
Sartorius MSC NutriStem® XF	Serum-free, xeno-free	hBMSC, hADSC, hWJMSC
STEMCELL Technologies MesenCult™-ACF Plus	Animal component-free, EV-free	hMSC
Kohjin Bio KBM ADSC-4	Serum free, xeno-free, chemically defined, phenol red-free, ready to use	Suitable for hADSC expansion
RoosterBio RoosterNourish™-MSC-XF	Xeno-free. cGMP version available. No surface coatings required when using Corning® CellBIND® surfaces.	hMSC, iPSC-MSC, immortalized MSC, and fibroblast
ROHTO Pharmaceutical R:STEM Medium	Animal component-free. No surface coatings required when using Corning CellBIND surfaces.	hMSC

Chapter 6: Importance of Surface Chemistry on Cell Culture Plasticware

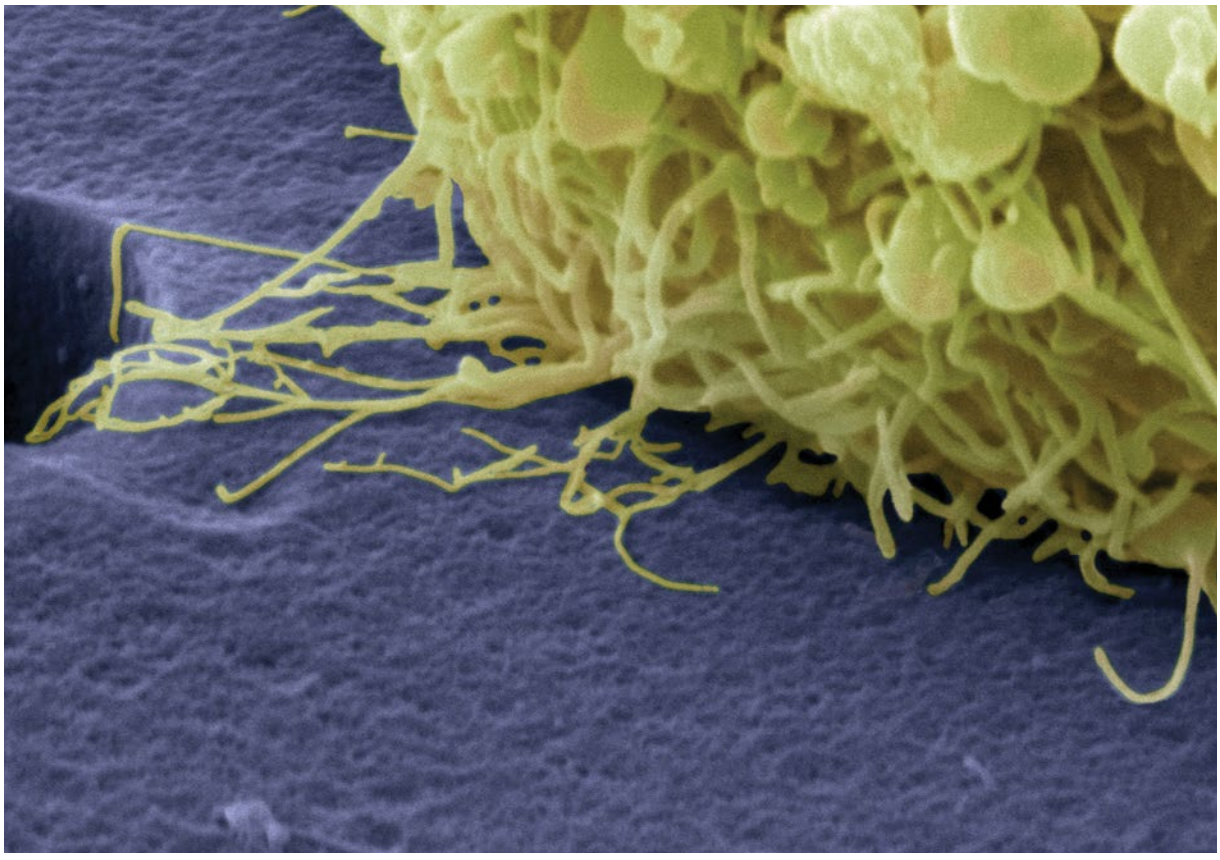
While conventional MSC culture methods involve using TC-treated plastic as a cell growth substrate, enhancing these surfaces with extracellular matrix (ECM) proteins like fibronectin, Collagen, vitronectin, or laminin can significantly improve cell attachment,proliferation, and multipotency. This improvement is evident in static culture conditions but becomes even more impactful in alternative cell expansion technologies, such as bioreactors or micro-carriers, where fluid flow is involved.

However, using biological coatings has its limitations, including time-consuming preparation, specialized storage and handling, and the high cost of quality ECM proteins for large-scale production. To overcome these challenges, alternatives such as the Corning CellBIND® surface technology has been developed. The CellBIND surface is an enhanced tissue culture treatment that creates a more hydrophilic and wettable surface, leading to improved cell spreading and attachment without the need for additional ECM coating. Implementing CellBIND technology in MSC culture can reduce costs and complications associated with ECM coatings while still maintaining high-quality MSCs.

The composition of proteins in the cell culture media directly impacts MSC attachment to a substrate. Therefore, selecting the preferred media formulation should be done in parallel with surface chemistry selection.

In the absence of serum, cells may struggle to adhere properly to traditional culture surfaces without the adhesion-promoting proteins found in FBS, such as fibronectin and vitronectin. Therefore, most serum-free media (SFM) require or recommend using costly serum-free adhesion substrates like CELLstart™ substrate (Thermo Fisher) or NutriCoat™ attachment solution (Sartorius) to optimize cell adhesion. As mentioned earlier, CellBIND surfaces support serum-free MSC culture by enhancing cell attachment more effectively than conventional TC-treated surfaces. For example, R:STEM medium (ROHTO Pharmaceutical) recommends using a CellBIND surface flask without ECM coating. Studies have also demonstrated that CellBIND surfaces support efficient MSC proliferation and retention of multipotency in SFM.

The combination of the CellBIND surface and SFM presents a powerful tool for researchers and bioproduction groups, providing more defined, consistent, and enhanced cell attachment in serum-free environments for cell production.



Chapter 7: Readily Available Cell Expansion Technologies From Corning

The number of required MSCs varies significantly depending on the application and whether an autologous or allogeneic therapy is being pursued. Often, isolating MSCs alone does not yield a therapeutically relevant number of cells, necessitating the use of expansion technologies to produce clinically significant quantities. The choice of expansion technology depends on several factors, but it is crucial to first determine your target cell number and work backward to ensure the selected technology can meet this goal.

As discussed, a key characteristic of MSCs is attachment to TC-treated plastic . Therefore, plastic-based cell culture vessels have traditionally been used for MSC research and scale-up. Several options are available to progress MSC culture beyond traditional, small-scale flatware, such as T-flasks and Petri dishes. However, additional considerations must be made when choosing a manufacturing platform. Key factors include:

- Scalability
- Operator training
- Contamination risk tolerance (open vs. closed system processing)
- Automation
- Process monitoring and control (PMC)
- Footprint
- Process development/optimization
- Labor costs
- Material costs for equipment (fixed upfront costs)
- Consumables (variable costs)

Given the adherent properties of MSCs, scale-up platforms are limited by the total available surface area. The cell expansion platforms are designed to provide a large cell growth surface area within a small volumetric footprint (i.e., surface area intensification). Broadly, stacked cell culture vessels, such as Corning CellSTACK® and HYPERStack® vessels, offer benefits like reduced requirements for operator training, process development/optimization, and equipment. However, they are offset by higher labor costs and fewer straightforward options for automation or PMC.

Alternatively, Corning CellCube® modules and polystyrene microcarriers paired with traditional bioreactors enable automation, PMC, and reduced labor but come with higher upfront equipment costs and more intensive training and process development/optimization requirements. For the described technologies, consistency of surface chemistry can be achieved at different scales with the availability of tissue culture and CellBIND surfaces. Detailed considerations for each platform are reviewed below.

Corning CellSTACK Vessels

CellSTACK vessels are stacked cell culture vessels that function similarly to traditional T-flasks, using the same core technology of cell attachment to a planar surface, static media conditions within an incubator, and gas exchange via a vented cap. CellSTACK vessels are available in 1-, 2-, 5-, 10-, and 40-layer options with 636 cm^2 of growth surface area per layer. This technology can be implemented as an open system or as a closed system using optional tubing assemblies. Minimal training and process development are required when scaling up from traditional flatware, making CellSTACK vessels one of the lowest barriers to entry for scale-up. A traditional CO_2 incubator is the only equipment required for MSC expansion in CellSTACK vessels. While academic-style incubators are suitable for small-scale processes, reach-in incubators are ideal for medium-scale processes. Most tissue culture labs already have the necessary infrastructure to implement this technology. At commercial production scales, the operational limitations and labor costs of handling hundreds of CellSTACK vessels can be substantially higher than for more intensified platforms. Therefore, CellSTACK vessels are often used in early-phase clinical manufacturing, for indications with a smaller patient population, or those requiring a low therapeutic dose. One technical limitation is the presence of a gas exchange gradient, where O_2 and CO_2 availability varies from the top to the bottom of the vessel. This is particularly relevant for the 40-layer CellSTACK vessel, which typically requires an active gassing strategy.

Corning HYPERFlask® and HYPERStack® Vessels

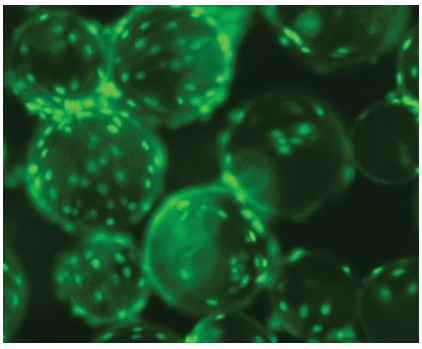
To avoid oxygen gradient limitations of CellSTACK vessels and further intensify the surface area, Corning developed the High Yield Performance (HYPER) technology. HYPERFlask and HYPERStack vessels are multi-layered and promote cell attachment to a static planar surface while maintaining optimal growth conditions within an incubator. HYPER technology addresses the gassing gradient challenge of traditional stacked vessels by incorporating a gas-permeable thin film of polystyrene as the cell growth surface, eliminating headspace, and offering more surface area in a given volumetric footprint. The HYPERFlask (1,720 cm²), HYPERStack 12-layer (6,000 cm²), and HYPERStack 36-layer (18,000 cm²) formats utilize the same core gas exchange technology. While the HYPERFlask vessel can be used for cell production, the HYPERStack vessel includes preassembled closed system tubing, facilitating usage in a manufacturing environment. Initial training on HYPERStack vessel manipulations is recommended, but operators can quickly learn the proper techniques to maximize cell seeding uniformity and harvest efficiency. Similarly to CellSTACK vessels, HYPERStack vessels only require an academic-style or reach-in incubator but may result in higher labor costs at very large scales. HYPERStack technology can be scaled out using tubing sets to seamlessly increase unit operations, offering reduced process development time and cost, while providing a scalable platform without significant capital investment.

Corning® CellCube® System

The CellCube system represents the next level of multi-layer vessel technology, maintaining the same tissue culture or CellBIND surface treatment. However, the CellCube system utilizes a separate media conditioning vessel, such as a controlled bioreactor, to offer improved process control and scalability. After cells attach to the CellCube module surface, a media recirculation loop is created by combining closed system tubing with a commercially available bioreactor and peristaltic pump. This strategy allows tight control of oxygen, pH, and temperature, optimizing the cell culture environment. The technology is available in 10-, 25-, and 100-layer options with 850 cm² of growth surface area per layer. Designed as a closed system, CellCube modules include pre-attached aseptic connectors that can be connected to various accessories for closed system processing. Varying degrees of automation, process monitoring, and control can be integrated depending on the choice of commercially available bioreactor and peristaltic pump paired with the CellCube module. As a semi-automated system, the CellCube system helps reduce labor requirements and improve scalability. Using the Corning CellCube product cart, four CellCube-100 modules can easily be connected in a single closed system totaling 340,000 cm² of surface area. The modular design enables even more CellCube modules to be connected in parallel and/or in series, when paired with an appropriately sized bioreactor and peristaltic pump, offering surface areas compatible with large scale processes.

Microcarriers

Microcarriers are small beads that can grow adherent cells in a suspension system such as a spinner flask, stirred tank, or vertical-wheel bioreactor. By combining many carriers into a single high-volume bioreactor, microcarriers offer tremendous scale-up potential and have been used in other biomanufacturing workflows (e.g., vaccine manufacturing) at the thousands of liters scale. Corning offers polystyrene microcarriers with surface treatments including Collagen, CellBIND (enhanced attachment), and Corning Synthemax®-II (a synthetic vitronectin peptide ideal for MSC culture). Each microcarrier is in the hundreds of micron diameter range and allows for cell attachment and spreading on the bead surface. Varying degrees of closed system processing, automation, process monitoring, and control are available for bioreactor systems. These features, combined with the scale potential, can drastically reduce labor requirements and improve productivity. However, developing microcarrier systems is challenging.



Pairing microcarriers with a bioreactor creates a complex and dynamic system that requires optimizing cell attachment to a floating particle, maintaining low shear during cell expansion, and achieving high cell harvest yield from the carriers. Often, conditions optimized at small scale do not translate to larger bioreactor systems, necessitating ongoing expert biological and engineering support to scale this technology. Unlike other technologies, microcarriers require additional processing to remove cells from the carrier surface. First, cells are removed using traditional enzymatic or non-enzymatic methods, but the efficiency of removal is often lower than for planar, 2D surfaces. Next, cells are separated from microcarriers using mechanical filtration, such as Corning cell strainers at small scale or Thermo Fisher Harvestainer™ bioprocess container at large scale.

Chapter 8: Considerations for Closed System and cGMP Manufacturing

Ensuring that any therapeutic is free from contaminating viral, bacterial, or fungal agents is crucial. This means that robust manufacturing process controls are essential to ensure a safe and effective product. These controls are built into the operational procedures of a cGMP (current Good Manufacturing Practice) manufacturing site and include cleaning procedures, documentation, raw materials testing, and personnel training. Ultimately, designing an MSC production process is all about risk reduction.

At the research scale, traditional cell culture handling processes are typically completed in a biosafety cabinet. However, transitioning to large-scale production requires rethinking how to maintain a sterile environment at scale in a clean room setting. This often involves the implementation of closed system solutions for aseptic processing. Such strategies use single-use sterile tubing and aseptic connectors to handle cell expansion, harvest, and downstream processes within a sterile fluid path.

When designing a closed system, several factors should be considered, including the clean room environment, operational fit, the volume to be transferred, and integration with various process steps. Ensuring that cellular production steps can interface seamlessly with downstream processes is essential. However, it may not be possible to close all process steps, so an individual risk assessment must be conducted to determine acceptable risk levels. This assessment should consider cross-contamination risks, operator exposure, clean room changeover times, clean room qualifications, and desired system flexibility.

A primary consideration for designing a closed system strategy includes selecting the optimal tubing and connectors that pair with the container or technology used for each process step. When choosing tubing for aseptic transfer, it is important to ensure the material is biocompatible and the tubing size is appropriate. Incorrect tubing material can lead to particulate contamination or absorption of the therapeutic product to the tube wall. Common tubing materials used in biopharmaceutical applications include:

- **Platinum-cured Silicone:** Preferred for its lower cost, favorable extractable and leachable profile, compatibility with peristaltic pumps for short-term pumping, and ability to be steam sterilized by autoclave. Platinum-cured silicone is not recommended for long-term pumping.
- **Thermoplastic Elastomer (TPE):** Ideal for heat welding and sealing, allowing sterile connections or disconnections. However, TPE is typically not used for pumping applications, apart from AdvantaFlex® tubing (AdvantaPure).
- **PharMed® BPT (Saint-Gobain):** More robust than silicone and suitable for longer-term pumping applications.

Making connections between different process steps requires careful consideration and rational design. Connections can be made by heat welding, which requires compatible tubing types and sizes, additional equipment, and time for each weld. Faster connections can be made with non-sterile connectors like Luer Lock and MPC connectors, which need to be handled in a biosafety cabinet. Aseptic connectors, such as CPC AseptiQuik®, Cytiva ReadyMate™ and Kleenpak™ connectors, allow for sterile connections in a non-sterile environment.

Volume transfer through a sterile closed system fluid path can be performed using gravity flow or a pump, often a peristaltic pump. For small to medium volume transfers, gravity flow is practical, but it requires the ability to place the transfer vessel above the receiving vessel, which can be challenging with large volumes or limited space. Using a pump provides additional process control and the ability to transfer larger volumes. It is important to select the appropriate pump type and compatible tubing to ensure proper flow rates and minimize shear stress on sensitive biological materials. Accessory clamps are frequently used to control liquid flow through different parts of the process.

Mapping out the entire process can be beneficial to determine the suitable containers, tubing, connectors, and volume transfer methods for each step of a bioproduction process.

Chapter 9: Cell Concentration and Buffer Exchange

Throughout the biomanufacturing process for MSCs, various steps will require cell concentration (volume reduction) and/or buffer exchange (washing) of a cell suspension. These steps can be employed at several stages, such as thawing a vial from a cell bank to remove DMSO, in between passages, after the final cell harvest, and before creating the final therapeutic dose. For example, after the final harvest of the full cell expansion volume, the cells need to be concentrated, and the growth media needs to be exchanged with a biologically inert buffer before the final fill and packaging. Each method involves reducing or replacing the liquid matrix in which cells are suspended.

- **Cell Concentration:** Reduces the volume of cell suspension without changing the composition of the liquid matrix.
- **Buffer Exchange:** Replaces the liquid composition with a fresh liquid matrix of the same or different composition.

In practice, cell concentration is often performed first, followed by resuspension in the new liquid matrix when a buffer exchange is required.

When selecting a cell concentration method, it is important to consider the required ranges of input and output volumes (often expressed as the turndown ratio), as well as the input and output cell numbers, processing time, optimization time, and closed system processing capability. It is also critical to choose a method that maintains a high number and proportion of viable and functionally active cells.

Centrifugation

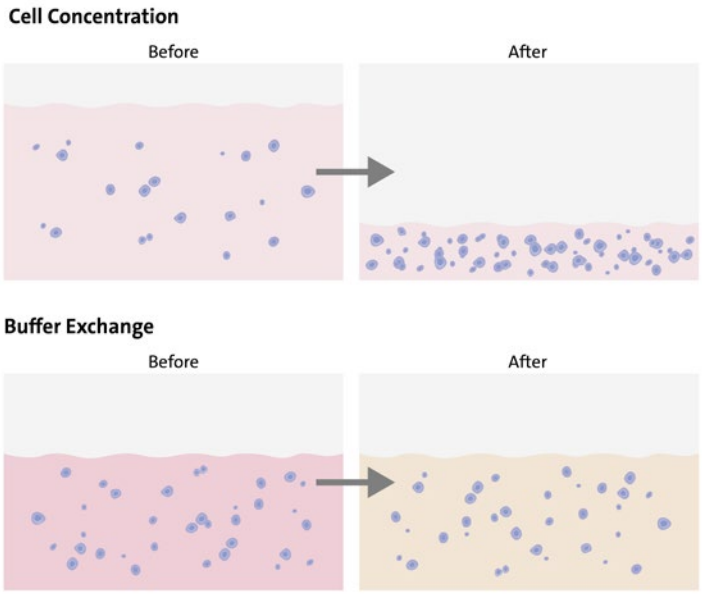
The classic method for MSC concentration and buffer exchange utilizes centrifugation, which separates cells from the less dense liquid matrix. This method typically involves manually processing conical tubes (such as Corning 50 to 500 mL conical tubes) in a traditional centrifuge. While centrifugation is simple and effective, it has limitations:

- Limited input volume (typically 15 to 500 mL)
- Requires open system processing, challenging to convert to a closed system
- Highly dependent on the technique of a skilled operator, introducing high run-to-run variability

Automated centrifugation-based technologies, such as the Cytiva Sefia™, Carr Biosystems UniFuge®, and GEA kytero®, have recently become available to address some of these limitations.

Filtration-based Methods

More recently, alternatives like filtration-based methods have been developed to improve upon centrifugation. The most common methods are tangential flow filtration (TFF) and alternating tangential flow filtration (ATF). These methods rely on single-use filters to mechanically separate cells, which are larger than the filter pores, from smaller components of the liquid matrix. Filtration-based technologies include the Fresenius Kabi Lovo® system, Repligen XCell® system, MilliporeSigma Cellicon™ assembly, and Verdot FlexiPro™ system.



Elutriation-based Methods

Elutriation-based methods combine traditional centrifugation with a fluid flow directed from the tip to the base of the packed cell bed, held within a conical centrifugation "cup." By independently selecting the centrifugation speed and fluid flow rate, cell selection can be finely tuned compared to traditional centrifugation. Elutriation-based technologies include Sartorius Ksep® system and Thermo Fisher Rotea™ system.

Both filtration- and elutriation-based methods offer closed system processing, automation, and large batch sizes, but each has limitations:

Filtration-based Methods: Prone to fouling of the filter or membrane, minimal options for selecting pore size to optimally select cells of a given size.

Elutriation-based Methods: Limited throughput by the size of the conical centrifugation cup, and the longer cells spend in the packed bed during elutriation, the more challenging it can be to recreate a single-cell suspension downstream.

Selecting a solution for cell concentration and/or buffer exchange depends on various factors and typically involves balancing cost and performance, such as cell recovery, processing time, and labor requirements. It is essential to consider the tunability of the chosen solution to compensate for fluctuations in input parameters, such as cell size and adhesiveness. For MSCs, tunability is even more critical due to the significant variation in these parameters based on the tissue source, donor, and batch of cells.

Chapter 10: Final Packaging, Freezing, and Storage

The final steps in the MSC biomanufacturing process involve careful selection of freezing solutions, packaging, and controlled freezing methods to ensure the drug product retains its quality attributes until it is delivered to the patient. While the container and freezing methods are important, the freezing solution is arguably more critical for maintaining the quality attributes of an MSC product. The chosen solution must protect the cells from damage during freezing and preserve their critical quality attributes (including high viability) when the cells are thawed for therapeutic delivery.

Freezing Solutions

Several prefabricated media formulations are available for freezing cells. Many of these options have known stability and storage data, a defined composition (drug master file), and are animal component-free (ACF). Examples of ACF cryopreservation media include Biolife Solutions CryoStor® and Sartorius NutriFreez® media. Injectable solutions such as PlasmaLyte A (Baxter) or Normosol-R® (Pfizer), supplemented with a cryoprotectant and human platelet lysate (hPL), serum, or plasma can also be used in cryopreservation solutions.

Packaging Selection

The selection of packaging should be based on the final filling volume and confirmed to be suitable for storage at the low temperatures required for a cell product. For small volumes and situations where an open system approach is acceptable, vials are typically used. Vials are often preferred for their simplicity, and they can be filled by hand or using an automatic fill and finish system. However, the filling steps must be completed in a grade A environment or biosafety cabinet.

When considering vials, the body and closure design are important. Classic screw-based cryogenic vials have the risk of screw failure at low temperatures. Glass vials, which can withstand low temperatures and have a favorable extractable and leachable profile, are another option, though the closure must be validated for low-temperature storage, which may be challenging.

For higher volumes and when a closed system filling is preferred, cryopreservation bags are often implemented. Selecting the correct bag material is crucial, as some types of plastic, like EVA, can become more rigid and crack at low temperatures. Corning cryopreservation bags, made from a special blend of polyolefin film and proprietary EVA, maintain flexibility and clarity at temperatures as low as -196°C.

Secondary Containers and Overwraps

For bags, a secondary container or overwrap must also be considered for storage. The overwrap provides protection against leakage and product loss. Cryopreservation bags typically have integrated TPE tubing with MPC or Luer Lock connectors. Connections between upstream process steps and the cryopreservation bag can be made manually using these connectors or via sterile welding. Automated fill and finish equipment, such as the Fresnius CUE®, Cytiva Sepax™ C-Pro, Comecer ValueCell, and Single Use Support RoSS.FILL system, can also be used to fill the bags.

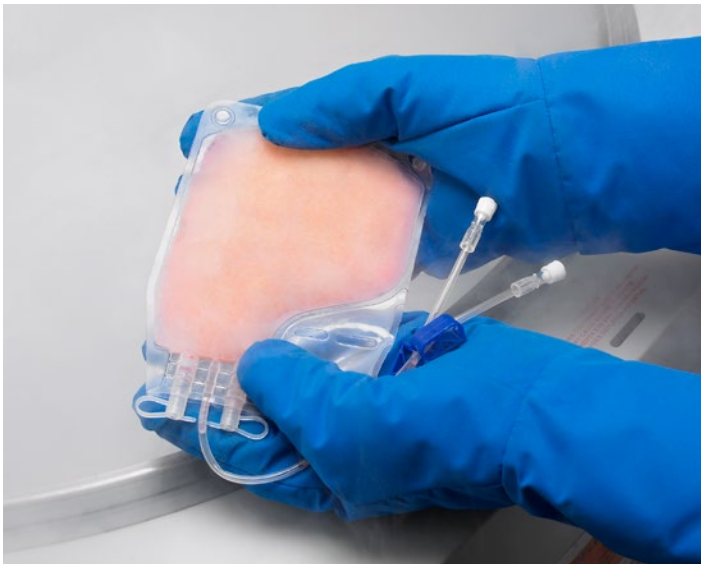
Freezing Methods

The rate at which cells are frozen impacts the formation of ice crystals that can damage cellular membranes. A typical target is a temperature drop of approximately -1°C per minute. Freezing methods can be categorized as passive freezing and controlled rate freezing.

Passive Freezing: This method uses a specially designed container with either isopropanol or a metal core (such as the Corning® CoolCell® container) to achieve a 1°C per minute temperature drop.

Controlled Rate Freezing (CRF): This method involves active temperature control using a controlled-rate freezer (CRF). Options include the Cytiva VIA Freeze™, Thermo Fisher CryoMed™, and Single Use Support RoSS. LN2 freezers. These solutions allow for the selection and design of a cryopreservation profile, control the temperature, and achieve temperatures below -150°C to freeze the cells.

Implementing the right combination of freezing solutions, packaging, and freezing methods is crucial to ensure the quality and viability of MSCs through the biomanufacturing process and until therapeutic delivery.



Chapter 11: Case Studies

To truly understand the life-cycle management of MSC manufacturing, it's invaluable to review case studies. The following examples illuminate important aspects of platform selection, culture methodologies, and the final assessment of the therapeutic properties of the generated material. We highlight three real-world situations that address critical parameters, bridging the gap between theoretical applications and practical situations. These case studies tackle different manufacturing needs and production expectations, including:

- Linear scalability
- Production space constraints
- Large-scale cGMP manufacturing requirements
- Production costs
- Shortening validation times

By examining these case studies, you can gain insights into how various challenges were overcome, ultimately leading to successful MSC manufacturing.

Case Study 1: Clinically Relevant Production of Bone Marrow-derived MSCs Using Corning® HYPERFlask® Vessels

From a healthcare system perspective, sepsis costs over $4 billion per year to treat. Despite decades of research, no targeted therapeutic agent for septic shock has improved clinical outcomes. Given the significant preclinical evidence, MSCs are considered a promising potential therapeutic option for this patient population. After numerous promising preclinical studies[8], the Regenerative Medicine Program at the Ottawa Hospital Research Institute (OHRI) took on the challenge of developing an MSC product for testing in a septic shock clinical trial.[9]

A primary consideration in cell therapy product development is ensuring process scalability without compromising the critical quality attributes of the cell product. Multi-layered Corning HYPERFlask cell culture vessels provided an attractive expansion platform due to their substantial surface area while maintaining a compact format. OHRI compared the expansion of clinical-grade MSCs from 2 donors using Corning T-175 flasks and HYPERFlask culture vessels with xeno-/serum-free culture medium. The expansion protocol involved an initial seeding of 1,000 cells/cm², cultivation for 6 days, harvesting with TrypLE™ enzyme, and analysis with the NC-200 NucleoCounter® (CFR Part 11/GMP-ready). Post-expansion, MSCs were characterized for surface marker profile according to ISCT criteria, trilineage differentiation potentials, and potency (inhibition of T-cell proliferation), as described previously.[10]

Results showed that MSCs expanded in T-175 flasks yielded 4.4 million and 4.5 million live cells from 2 different donors. In contrast, MSCs expanded in HYPERFlask vessels produced 43 million and 49 million cells from the same donors. Cell proliferation rates were comparable between the two vessels, as MSC doubling times and total fold expansion in T-175 flasks and HYPERFlask vessels were similar. The difference in cell yields is attributable to the available surface area for cell expansion. A HYPERFlask vessel has a 9.8-fold higher surface area compared to a T-175 flask, resulting in 9.8X and 11.0X higher cell yields for the two donors, respectively.

Post-expansion characterization revealed that the MSCs maintained their surface marker phenotype (positive for CD73, CD90, and CD105, and negative for CD14, CD19, CD34, CD45, and HLA-DR), retained the capacity to differentiate into adipocytes, chondrocytes, and osteocytes, and exhibited potency to inhibit T-cell proliferation. The use of HYPERFlask vessels resulted in an approximately 10-fold greater MSC yield compared to conventional T-flask culture, demonstrating the linear scalability of the available surface area of the vessel.

Post-harvest Analysis

	Surface Area	MSC Source	Cell Yield (Total)	Cell Yield	CD73/90/109	CD14/19/34/45, HLA-DR
T-175 flask	175 cm^2	Donor 1	4.4 million	25.1 K/cm^2	Positive	Negative
		Donor 2	4.5 million	25.7 K/cm^2	Positive	Negative
Corning HYPERFlask vessel	1,720 cm^2	Donor 1	43 million	25.0 K/cm^2	Positive	Negative
		Donor 2	49 million	28.5 K/cm^2	Positive	Negative

Case Study 2: Manufacturing MSC Therapeutics in Corning® HYPERStack® Vessels

The use of MSCs for transplantation therapy for spinal cord and muscular injuries has been well characterized. Biotechnology companies like CryoVida Biotech have been providing regenerative therapies for over a decade using endometrium-derived MSCs isolated from 4 donors. The challenge for Cryovida Biotech and similar therapeutic providers is to continue supporting growing patient demand within existing production settings.

Previously, Cryovida Biotech utilized 5-layer Corning CellSTACK® vessels to manufacture MSCs from 4 different donors, typically yielding an averageof 258 million cells per unit. Due to increasing patient demand, they needed to improve the scalability of their manufacturing process. Cryovida Biotech implemented Corning HYPERStack technology, which increased their typical average yield to 1.6 billion cells per 36-layer HYPERStack vessel. Furthermore, they adopted a 4-unit, 36-layer HYPERStack manifold closed system production process to boost efficiency. This allowed Cryovida Biotech to increase their monthly manufacturing capacity to 6-7 billion cells per batch while using the same equipment, personnel, and manufacturing space.

Post-harvest Analysis

	Surface Area	MSC Source	Cell Yield (Total)	Cell Yield	CD73/90/109	CD14/19/34/45, HLA-DR
5-layer Corning CellSTACK vessel	3,180 cm^2	Donor 1	170 million	53.4 K/cm^2	Positive	Negative
		Donor 2	150 million	47.1 K/cm^2	Positive	Negative
		Donor 3	148 million	46.5 K/cm^2	Positive	Negative
		Donor 4	190 million	59.7 K/cm^2	Positive	Negative
36-layer HYPERStack vessel	18,000 cm^2	Donor 1	1050 million	58.3 K/cm^2	Positive	Negative
		Donor 2	1100 million	61 K/cm^2	Positive	Negative
		Donor 3	1200 million	66.6 K/cm^2	Positive	Negative
		Donor 4	990 million	55 K/cm^2	Positive	Negative

Case Study 3: Effective Manufacturing of Allogeneic Mesenchymal Stem Cells Using a Scalable Closed System Circulation Platform

S-Quatre Corporation, a Japanese biotechnology company affiliated with Kidswell Bio Corporation, specializes in regenerative medicine. The company primarily focuses on developing cell therapies using dental pulp stem cells isolated from baby teeth, known as SHED (stem cells from human exfoliated deciduous teeth). SHED have a high intrinsic growth capacity and can produce various trophic factors, capabilities that are further enhanced by S-Quatre's proprietary culture methods, through which the resulting cells are named SQ-SHED. Their research aims to treat various difficult-to-cure diseases by harnessing the regenerative potential of SQ-SHED, which is currently in clinical development for cerebral palsy and in preclinical stage for bone disorders and brain tumors.

S-Quatre has developed a stable supply system, branded as S-Quatre®, for donor recruitment, banking, and supply of these cells, providing a solid foundation for cell therapy production. Thanks to the high proliferative potential of SQ-SHED, a single donor tooth can yield tens of billions on injectable doses through a two-tier cell banking system comprising a Master Cell Bank and a Working Cell Bank. To realize this approach, they explored various technologies to scale the manufacturing of SQ-SHED, aiming to produce billions to trillions of cells per batch. Traditional stacked vessel technologies posed challenges due to high demands on space and labor. Therefore, S-Quatre sought a new platform that could meet their scalability needs, provide process controls, and increase efficiency for manufacturing high-quality cells.

SQ-SHED yields in the Corning CellCube® system were compared to the legacy expansion process using Corning CellSTACK® vessels. The CellCube system achieved equivalent cell densities and population doubling times compared to CellSTACK vessels, and the harvested cells were confirmed to express MSC markers, ensuring their characteristics and quality. Metabolic analysis showed high scalability from the CellCube 25-layer to the CellCube 100-layer, supporting feasible scale-up development. More importantly, approximately 65% less media was required due to the process control features provided by the bioreactor.

Post-Harvest Analysis

	Surface Area	MSC Source	Cell Yield (Total)	Cell Yield	CD73/90/105	CD14/19/34/45, HLA-DR
2-layer CellSTACK vessel	1,272 cm^2	Single Donor	55 million	44 K/cm^2	Positive	Negative
100-layer CellCube system	85,000 cm^2	Single Donor	3.8 billion	45 K/cm^2	Positive	Negative

Conclusions

The production of mesenchymal stem cells (MSCs) for therapeutic applications involves a complex and multi-faceted process that spans from initial isolation to large-scale expansion and final product formulation. Throughout this whitepaper, we have explored various critical aspects of MSC manufacturing, including isolation methods, media formulations, surface chemistry, cell expansion technologies, and cryopreservation techniques. By examining real-world case studies, we have highlighted the importance of platform selection, culture methodologies, and maintaining the therapeutic properties of MSCs to meet the growing demand for these promising cell-based therapies.

Key takeaways include:

- **Scalability and Efficiency:** Implementing advanced technologies such as the Corning® HYPERFlask®, HYPERStack® vessels, and CellCube® systems can significantly enhance the scalability and efficiency of MSC production, allowing for the generation of billions to trillions of cells while maintaining critical quality attributes.
- **Media and Surface Chemistry:** The choice of media and surface chemistry plays a crucial role in ensuring optimal cell attachment, proliferation, and maintenance of MSC multipotency. Transitioning to serum-free, xeno-free, and animal component-free media formulations can reduce variability and safety concerns, making them more suitable for clinical applications.
- **Closed System Processing:** Adopting closed system solutions for aseptic processing minimizes contamination risks and streamlines the workflow, especially when scaling up to large-scale production. These systems, combined with appropriate tubing, connectors, and automated fill and finish equipment, facilitate a sterile environment throughout the manufacturing process.
- **Cryopreservation:** Selecting the right freezing solutions and packaging, along with a controlled freezing method, is essential to maintain cell viability and functionality during storage and transport. Prefabricated media formulations and advanced cryopreservation bags and vials provide reliable options for preserving MSCs.
- **Process Optimization:** Continuous process optimization, including monitoring and controlling critical parameters such as pH, dissolved oxygen, and cell health status, is vital for producing high-quality MSCs. Advanced bioreactor systems and process control features can help achieve these goals efficiently.

To further support our customers in navigating these complexities, the Corning Field Application Scientist (FAS) team offers extensive expertise and assistance in cell-based manufacturing and scale-up. The FAS team, is dedicated to partnering with customers for process development and optimization. We provide a range of services, including seminars, detailed instructions, protocol development, troubleshooting, process consultation, hands-on training, and on-site validation.

Whether you are optimizing open vs. closed systems, integrating automation solutions, or fine-tuning gassing strategies, our team is here to help. We also offer expertise in biological optimization, such as process parameter control, surface chemistry selection, and cell health monitoring, to ensure the highest quality MSC production.

In conclusion, the successful production of MSC-based therapies requires a combination of advanced technologies, meticulous process control, and expert guidance. By leveraging the expertise of the Corning FAS team, you can achieve optimal results at every stage of MSC manufacturing, ultimately delivering high-quality, scalable, and effective cell therapies to patients in need.

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