MANUFACTURING AND TESTING

Our product is Wharton's Jelly derived mesenchymal stem cell exosomes. It is a cell free product which we package with two variants, one of them is sterile aseptic filled saline product with 20 billion exosomes per cc.

Once we get the umbilical cord we move it to our ISO-5 Clean Room for processing. We will only harvest our stem cells from a fresh umbilical cord, never froze. This is important because the freezing of bulk tissue causes damage to the cells which causes major differences in stem cell behavior and makes it very difficult to work with. Once we harvest the MSC stem cells we run them through some **CNC** as well as **performative testing**. This is to ensure the stem cells are as characterized and they are actually mesenchymal stem cells. Additionally, we do this to find out the responsiveness of the stem cells and the underlying mechanism of action. The goal isn't just to have "exosomes", the goal is to have exosomes that contain high concentrations of key growth factors and anti-inflammatory factors and that the exosome structure itself is the type that will target the damaged and inflamed tissue.

We do **preliminary testing** to find out what the responsiveness of the stem cell is and how they are going to behave. Based on those results we then determine whether we are going to use the first, second, or third passage of the cell line. Every time you expand the cells and grow them out, they reduce their responsiveness, and the total concentration and potency of the product will decline every generation of stem cells. So, we only use up to the third generation and that is based entirely on performative measures of the stem cells. Once we have that determined we will create a **master cell bank** for that tissue and then send that out for testing through a 3rd party laboratory. Once the master cell bank testing comes back clean, we then go into production. We are required to make a consistently potent product with every batch and to accomplish this, every day we stimulate the stem cells by giving them **specialized culture media** that we developed in house which is xeno-free so there are **no animal products or foreign substances** in it. Additionally, it ensures that the stem cells do not multiply, divide or differentiate in any way shape or form while they are in the bioreactors. We take **daily measures** of the production of our stem cells in house and then based on the results, we adjust the food source and the stimulating factors to ensure that the finished product that we have at the end of a batch manufacturing conforms with the 351(a) consistency standards. So, we are able to produce the **exact same drug** today as we will thirty years from now even when using different tissue. Our end product **always has the highest potency that you can get out of stem cells** so the concentration of key growth factors and anti-inflammatory factors per exosome is much higher than anything you will find with the standard products on the market today.

Once that process is complete, we concentrate that solution one thousand fold and we send that out for **batch virology testing**. Once those results come back clean, we then go into finish product manufacturing where we take that concentrate to 20 billion exosomes per cc along with standard 0.9% saline. Next, we send 25% of our product out for **finished lot analysis by a 3rd party laboratory** as we do with all our testing. The lab will then perform **sterility, endotoxin and mycoplasma** testing that meets the safety standards followed up with **proteomics, mRNA sequencing and lipid analysis studies** of both the free floating as well as loaded within the exosomes, so you get two concentrations. One is what is in the total vial and the other is how much is in each exosome. We measure what is inside of the exosomes once in the vial. Once that is done, we also perform an analysis on quantity of exosomes through particle size analysis as well as the type and function of the exosomes through **protein analysis**.

TYPES OF TESTS EXPLAINED

What is Batch Concentrate Virology Testing for Mesenchymal Exosomes?
When making mesenchymal exosomes, it's super important to make sure they're safe to use. One big safety check is called **batch concentrate virology testing**. Let's break that down:

What Are Mesenchymal Exosomes?
Mesenchymal exosomes are tiny, bubble-like particles that come from special cells called **mesenchymal stem cells (MSCs)**. These exosomes carry important signals and growth factors that can help with healing and reducing inflammation.

What Does "Batch" Mean?
A **batch** is just a large group of exosomes that are made at the same time in the lab. Think of it like baking a big batch of cookies—everything made in that batch is mixed and prepared together.

What is "Concentrate"?
When scientists make exosomes, they often need to **concentrate** them. This means they gather a lot of exosomes into a smaller amount of liquid. This makes the treatment stronger and more effective.

What is Virology Testing?
Virology testing checks for any harmful **viruses** that might be in the exosome batch. Viruses are tiny germs that can make people sick, so it's super important to make sure there aren't any in the exosomes.

Putting It All Together:
When scientists do **batch concentrate virology testing**, they are:

1. **Testing the entire batch** of exosomes to make sure they are safe.
2. **Checking for viruses** in the concentrated exosome liquid.
3. Making sure that no harmful viruses are present before the exosomes are used for treatments.

Why Is This Important?
By doing this testing, doctors and scientists make sure that the exosomes are safe for patients. It helps prevent infections and ensures the treatment is clean and effective.

What is Proteomics Testing for Mesenchymal Exosomes?
Proteomics testing is a process that scientists use to study all the **proteins** found in mesenchymal exosomes. Proteins are like tiny tools or messengers in the body that help cells communicate, heal, and perform everyday functions.

What Does Proteomics Testing Do?

Proteomics testing looks at the full set of proteins (called the **proteome**) within a batch of mesenchymal exosomes. This testing helps answer important questions like:

- **What proteins are present?** It identifies specific proteins carried by the exosomes.

- **How much of each protein is there?** It measures the concentration of each protein.
- **What do these proteins do?** It analyzes the role of these proteins in healing and cell communication.

Why is Proteomics Testing Important for Mesenchymal Exosomes?

1. **Ensures Quality:** By knowing exactly which proteins are present, manufacturers can ensure consistency and quality in each batch of exosomes.
2. **Demonstrates Potency:** Certain proteins are linked to healing properties, so testing can show how effective the exosomes might be.
3. **Safety Checks:** It helps detect any unwanted proteins that could cause side effects.
4. **Customizing Treatments:** Understanding the protein profile allows for more targeted therapies, especially for specific medical conditions.

How is Proteomics Testing Done?
The testing process often involves:

1. **Extraction:** Collecting the proteins from the exosome samples.
2. **Separation:** Using techniques like **mass spectrometry** to separate and identify proteins.
3. **Analysis:** Comparing the proteins found to a database of known proteins to understand their functions.

Example:

If a batch of mesenchymal exosomes is intended for use in treating inflammation, proteomics testing might show high levels of anti-inflammatory proteins, confirming the treatment's potential effectiveness.

Bottom Line:
Proteomics testing is like creating a detailed **inventory list** of all the proteins in mesenchymal exosomes. It helps ensure the treatment is safe, consistent, and powerful for healing and regeneration.

What is a Master Cell Bank (MCB)?

A **Master Cell Bank** is a large collection of mesenchymal stem cells that have been thoroughly tested and stored in a controlled environment. These cells serve as the **"starting material"** for all future production of mesenchymal exosomes.

- **Think of it like this:** The MCB is the “seed” that will produce many “fruits” (exosomes). By ensuring the seed is perfect, all the fruits will be consistently good.

What Does Master Cell Bank Analysis Involve?

1. Cell Identity Testing:

- Confirms that the cells in the MCB are truly **mesenchymal stem cells**, often through **flow cytometry** and **surface marker analysis**.

2. Purity Testing:

- Ensures that no **contaminants**, such as bacteria, fungi, viruses, or other unwanted cell types, are present.

3. Viability Testing:

- Checks that the cells are alive and healthy, ensuring they can produce high-quality exosomes.

4. Genetic Stability Testing:

- Evaluates whether the cells maintain a **stable genetic profile** over time, reducing the risk of mutations.

5. Potency Testing:

- Measures the functional ability of the MSCs to produce exosomes with **desired therapeutic properties**, such as promoting healing or reducing inflammation.

Why is Master Cell Bank Analysis Important?

1. **Ensures Safety:**
   Detects and prevents harmful contaminants or unwanted cell types.
2. **Maintains Consistency:**
   Provides a **standardized source** of MSCs, so every batch of exosomes has the same quality and potency.
3. **Regulatory Compliance:**
   Meets strict guidelines set by regulatory bodies like the **FDA** or **EMA**, which require thorough testing of cell-based therapies.
4. **Reduces Risk:**
   Helps avoid complications like **immune reactions** or **ineffective treatments** by ensuring the exosomes are derived from well-characterized and stable cells.
5. **Supports Efficacy:**
   High-quality MSCs lead to **high-quality exosomes**, which can improve patient outcomes in treatments for conditions like **inflammation**, **neurological disorders**, or **tissue regeneration**.

In Simple Terms:

Master Cell Bank analysis is like **quality control at the source**. It makes sure that the "factory" (the stem cells) making the "products" (the exosomes) is running smoothly, safely, and consistently. By starting with the best cells, every batch of exosomes produced will be reliable and effective for therapeutic use.

What is Flow Cytometry and Surface Marker Analysis for Mesenchymal Exosomes?

When producing **mesenchymal exosomes**, it's essential to ensure their quality, safety, and effectiveness. **Flow cytometry** and **surface marker analysis** are advanced laboratory techniques used to analyze both **mesenchymal stem cells (MSCs)** and the **exosomes** they produce.

1. What is Flow Cytometry?

Flow cytometry is a powerful tool used to analyze cells or particles (like exosomes) by measuring their physical and chemical properties.

How Does It Work?

- **Sample Preparation:** The sample, which may include MSCs or exosomes, is mixed with special **fluorescent markers** that bind to specific proteins on the cell or exosome surface.
- **Flowing Through a Laser:** The sample is passed through a narrow tube where each cell or particle goes through a **laser beam**.
- **Light Detection:** When the laser hits the fluorescent markers, it produces light signals.
- **Data Analysis:** The signals are captured and analyzed by a computer, providing information about **size**, **shape**, and **surface proteins** of the particles.

Why is It Important for Exosomes?

- **Identifies Exosome-Specific Markers:** Helps confirm that the particles are genuine exosomes (e.g., expressing markers like **CD9**, **CD63**, **CD81**).
- **Quality Control:** Ensures the exosome preparation is pure and not contaminated with other particles or cell debris.
- **Quantification:** Measures the number of exosomes present in a sample, which is important for dosing and therapeutic applications.

2. What is Surface Marker Analysis?

Surface marker analysis involves examining specific proteins present on the outer membrane of MSCs or exosomes. These proteins, called **surface markers**, act like **ID tags**, providing critical information about the cell type and the exosome's potential function.

For Mesenchymal Stem Cells (MSCs):

- **Common MSC Markers: CD73**, **CD90**, **CD105** (positive markers), and **CD45**, **CD34**, **CD14**, **CD19**, **HLA-DR** (negative markers).
- **Purpose:** Confirms that the cells are indeed MSCs and not contaminated with other cell types.

For Mesenchymal Exosomes:

- **Common Exosome Markers: CD9**, **CD63**, **CD81**, **TSG101**, **Alix**.

- **Purpose:** Validates that the isolated particles are true exosomes and helps assess their therapeutic quality.

Why Are These Tests Important in the Context of Mesenchymal Exosomes?

1. **Ensures Purity:** Helps detect and eliminate contaminants, such as unwanted cells, proteins, or particles.
2. **Validates Identity:** Confirms that the product is genuinely composed of MSCs and their exosomes.
3. **Supports Consistency:** Standardizes exosome production by verifying that every batch meets the same quality standards.
4. **Regulatory Compliance:** Many health authorities require this level of testing to approve biological products for clinical use.
5. **Enhances Safety:** Minimizes the risk of adverse reactions in patients by ensuring the exosome product is well-characterized and pure.

In Simple Terms:

- **Flow cytometry** is like a high-tech scanner that examines each tiny particle in a sample, telling us what's inside and if it's the right stuff.
- **Surface marker analysis** helps us identify these particles by reading their "ID tags," ensuring that only the right types of exosomes are being used for treatments.

Together, these tests act as a **quality checkpoint**, ensuring that the mesenchymal exosomes are safe, effective, and ready for therapeutic use.

Why is Sterility Testing Important for Mesenchymal Exosomes?

1. **Patient Safety:** Prevents the risk of infections when exosomes are administered to patients.
2. **Regulatory Compliance:** Required by health authorities like the **FDA** and **EMA** to ensure the safety and quality of biologic products.
3. **Product Integrity:** Maintains the **purity** and **potency** of the exosome product.
4. **Clinical Efficacy:** Ensures that the therapeutic benefits of exosomes are not compromised by contamination.

The Sterility Testing Process for Mesenchymal Exosomes:

1. Sample Collection:

- A small sample of the final exosome product is collected in a **sterile environment** to avoid accidental contamination.

2. Culture-Based Sterility Testing:

- The sample is placed in **nutrient-rich media** that encourages the growth of any potential microorganisms.
- The media are typically incubated at specific temperatures for **14-21 days**, providing ideal conditions for microbial growth.
- Two types of media are commonly used:
    - **Thioglycollate broth:** For detecting **anaerobic** (oxygen-free) microorganisms.
    - **Tryptic soy broth:** For detecting **aerobic** (oxygen-requiring) microorganisms.
- If no growth (e.g., cloudiness or colonies) is observed, the product is considered **sterile**.

3. Rapid Sterility Testing:

- In addition to traditional culture methods, rapid tests using **molecular techniques** like **PCR (Polymerase Chain Reaction)** can detect microbial DNA quickly, often within **24-48 hours**.
- This method is useful for faster product release while maintaining safety.

4. Endotoxin Testing (Optional but Recommended):

- **Endotoxins** are toxic substances released from the outer membrane of certain bacteria, even if the bacteria are not alive.
- The **LAL (Limulus Amebocyte Lysate) test** or **Recombinant Factor C (rFC) test** is used to detect endotoxins.
- Ensuring **low endotoxin levels** is critical, as high levels can cause severe inflammatory responses in patients.

5. Mycoplasma Testing:

- **Mycoplasma** are tiny bacteria that can contaminate cell cultures and exosome products.
- Special tests, including **culture methods** or **molecular tests (e.g., PCR)**, are conducted to ensure that the exosomes are **mycoplasma-free**.

Quality Control Measures:

- **Environmental Monitoring:** The manufacturing facility is regularly checked for cleanliness and sterility.
- **Aseptic Techniques:** All processes are conducted in **sterile conditions**, often in **clean rooms** with **HEPA filtration**.
- **Batch Testing:** Every batch of exosomes undergoes sterility testing before being released for clinical or therapeutic use.

What Happens if Contamination is Found?

- If sterility testing detects contamination, the entire batch of exosomes is **discarded**.
- The manufacturing process is reviewed to identify and fix the source of contamination before producing a new batch.

In Simple Terms:

Sterility testing is like a **safety check** to make sure mesenchymal exosomes are **clean and safe**. Scientists grow samples of the exosomes in special conditions to see if any germs appear. If no germs grow, the exosomes are considered **safe to use** in medical treatments.

What is mRNA Sequencing for Mesenchymal Exosomes?

mRNA sequencing (also known as **transcriptome sequencing**) is a technique used to analyze the **messenger RNA (mRNA)** molecules present within **mesenchymal exosomes**. This process helps scientists understand which **genes** are active and what **biological messages** the exosomes are carrying.

What are Mesenchymal Exosomes?

Mesenchymal exosomes are tiny, bubble-like particles released by **mesenchymal stem cells (MSCs)**. They act as **cellular messengers**, carrying important molecules such as **proteins**, **lipids**, and **RNA**, including **mRNA**, to other cells. These exosomes play a significant role in **cell-to-cell communication**, promoting **healing**, **regeneration**, and **reducing inflammation**.

What is mRNA and Why is it Important?

mRNA (messenger RNA) is a type of RNA that carries **genetic instructions** from DNA to the cell's machinery to produce specific **proteins**. In the context of mesenchymal exosomes, mRNA can:

- Deliver therapeutic signals to **target cells**.
- Influence **cell behavior**, such as promoting **tissue repair** or **modulating the immune system**.
- Provide insights into the **biological functions** of the exosomes.

The mRNA Sequencing Process for Mesenchymal Exosomes:

1. Isolation of Exosomes:

- Mesenchymal exosomes are first **isolated** from cell culture media or biological fluids using techniques like **ultracentrifugation**, **size exclusion chromatography**, or **precipitation methods**.

2. Extraction of RNA:

- The exosomes are **lysed** (broken open) to release their **contents**, including mRNA.
- The **mRNA** is then extracted and purified from the total RNA.

3. Preparation of RNA Library:

- The mRNA is converted into **complementary DNA (cDNA)** using a process called **reverse transcription**.
- **Adapters** are added to the cDNA fragments to prepare them for sequencing.

4. Sequencing:

- The cDNA is sequenced using advanced technologies like **Next-Generation Sequencing (NGS)**.
- This generates a **detailed profile** of all the mRNA molecules present in the exosomes.

5. Data Analysis:

- The sequencing data is analyzed using bioinformatics tools.
- Scientists identify which genes are expressed and quantify the levels of different mRNA transcripts.
- The analysis can reveal specific biological pathways influenced by the exosomes.

Why is mRNA Sequencing Important for Mesenchymal Exosomes?

1. **Understanding Therapeutic Potential:**
    - Identifies key mRNA molecules involved in healing, anti-inflammatory effects, and tissue regeneration.
2. **Quality Control:**
    - Ensures the exosomes contain consistent and beneficial mRNA profiles, which is crucial for clinical applications.
3. **Mechanism of Action:**
    - Helps explain how exosomes work at the molecular level, providing evidence to support therapeutic claims.
4. **Biomarker Discovery:**
    - Detects specific mRNA signatures that can serve as biomarkers for monitoring treatment efficacy.
5. **Regulatory Compliance:**
    - Provides data needed to meet regulatory requirements for the safety and effectiveness of exosome-based therapies.

Example:

If a batch of mesenchymal exosomes is intended for treating **neurodegenerative diseases**, mRNA sequencing might show **high levels of mRNA** that promote **neuronal survival** and **anti-inflammatory responses**. This insight can help tailor the exosome product to **maximize therapeutic benefits** for patients with conditions like **Parkinson's disease** or **Alzheimer's disease**.