Hollow Fiber Bioreactors and Cytocentricity: Applying Cytocentric Principles

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The concept of the cytocentric bioreactor was introduced by a regenerative medicine consortium to promote a more "cell-centric" perspective in cell culture systems. Traditional 2-D and low-density suspension culture methods are inherently non- physiologic, suffering from high variability and inconsistent post-translational modifications and protein folding. These methods often lead to significant contamination from host cell proteins and DNA. The cytocentric bioreactor emphasizes functionality from the cell's perspective, aiming to replicate the *in vivo* environment within in vitro systems. To enhance reproducibility and efficacy of biologically derived therapies, five cytocentric principles have been proposed:

Cells need:

1) Protection from microbial contamination
2) Physiologic stimulation
3) Full-time optimal conditions
4) Individualized conditions
5) Dynamic environments as populations evolve

The Shortcomings of People-Centric Cell Culture Practices

Current cell culture practices are best described as "people-centric." Laboratory facilities are generally designed for the comfort and efficiency of the workers. Benches, chairs,

hoods, and pipettors in the lab are “people-centric,” designed by people for people. Even a CO2 incubator is designed for the needs of people in the lab, not the cells. It is also prone to contamination, with supraphysiologic O2 levels and fluctuating CO2 and temperature each time the door opens. Yet, the standard room air CO2 incubator persists because it is familiar, cheap, and easy to use—for people. Like people, cells have design requirements for optimum efficiency and reproducibility.

Cells are routinely taken out of incubators and transported to cell handling spaces or machinery, where they encounter conditions that vary significantly from the incubator and deviate from their physiologic needs. Traditional people-centric cell culture practices prioritize convenience over the optimal conditions required by cells.

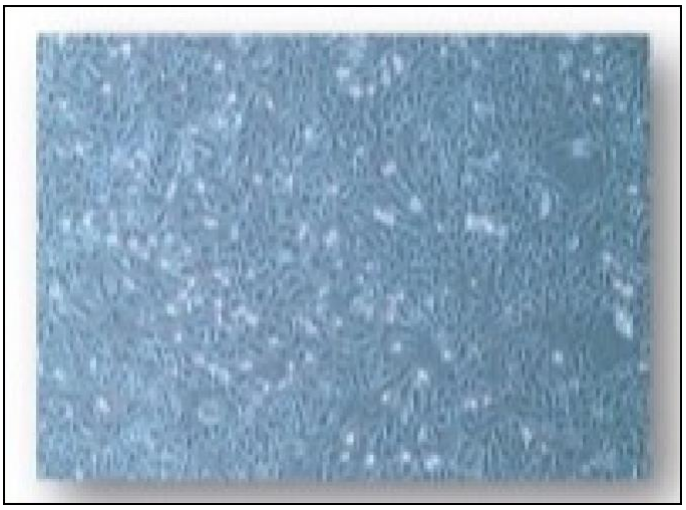


Figure 1: Cells grown as a monolayer in 2-D plastic flasks is not reflective of how they grow *in vivo.*

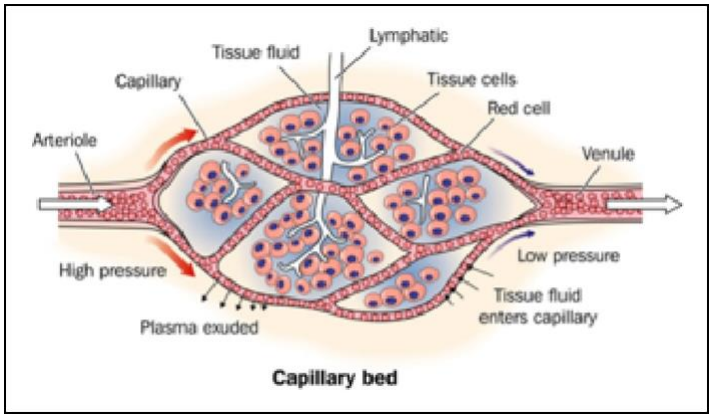


Figure 2: Graphic representation of the 3-D capillary bed, the ultimate cytocentric bioreactor.

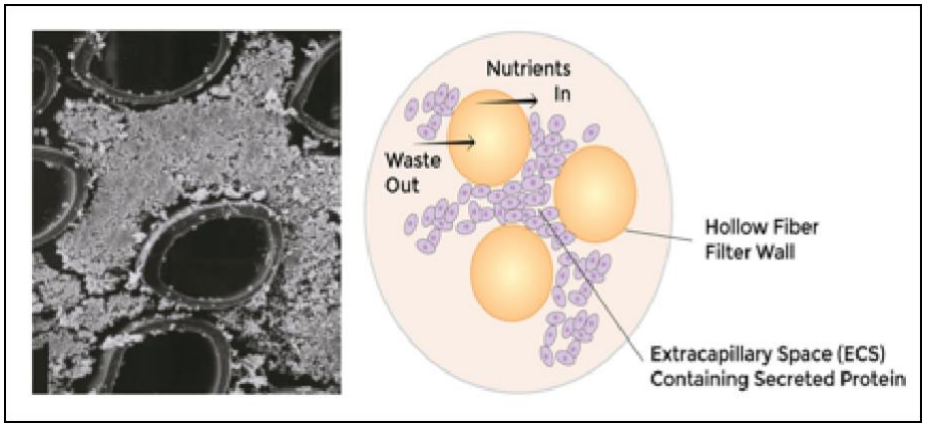


Figure 3: Cross section of a hollow fiber bioreactor (photo and diagram) demonstrating how it closely approximates the 3-D geometry of the capillary bed.

Principles of Cytocentric Bioreactors

1. Cells Need Protection from Microbial Contamination

The invention of the laminar flow cleanroom by Willis Whitfield in 1960 revolutionized semiconductor and pharmaceutical research and manufacturing by creating particle-free environments. These cleanrooms led to the development of laboratory laminar flow hoods in the early 1970s. Despite advancements, culturing cells in 2-D plastic flasks with fetal bovine serum remains common, though it is increasingly recognized as non- physiologic.

The environment that supports cells in culture is also an ideal environment for the growth of microbial contaminants. Antibiotics can mask small-scale infections and alter gene expression, impairing cell growth and differentiation. A cytocentric bioreactor, such as a hollow fiber bioreactor, operates as a closed system, protecting cultures from contamination and shielding operators from biohazardous materials. Studies have shown that closed systems like hollow fiber bioreactors reduce contamination rates significantly compared to traditional open systems.

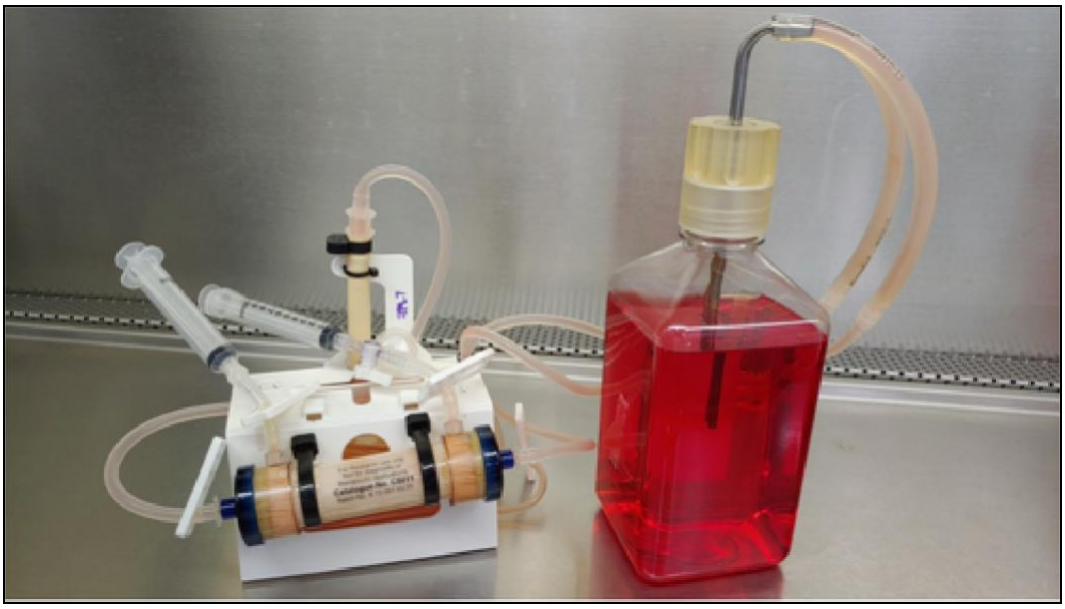
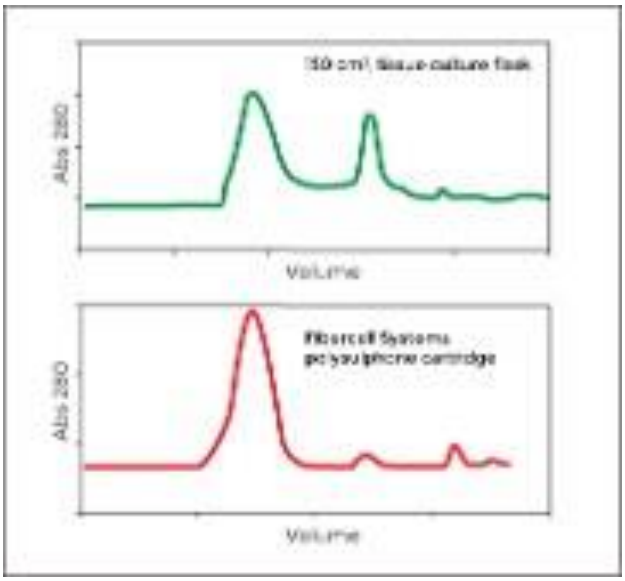


Figure 4: The FiberCell hollow fiber bioreactor is a completely closed system providing protection for the cells from microbial contamination and protection for the operator when working with infectious pathogens.

2. Cells Need Physiologic Stimulation

Uncontrolled people-centric laboratory conditions are not physiologic nor reproducible from site to site. Proper cell culture conditions can result in complete and uniform post- translational modifications and protein folding. Providing more cytocentric conditions for cells in culture improves biomedical reproducibility.



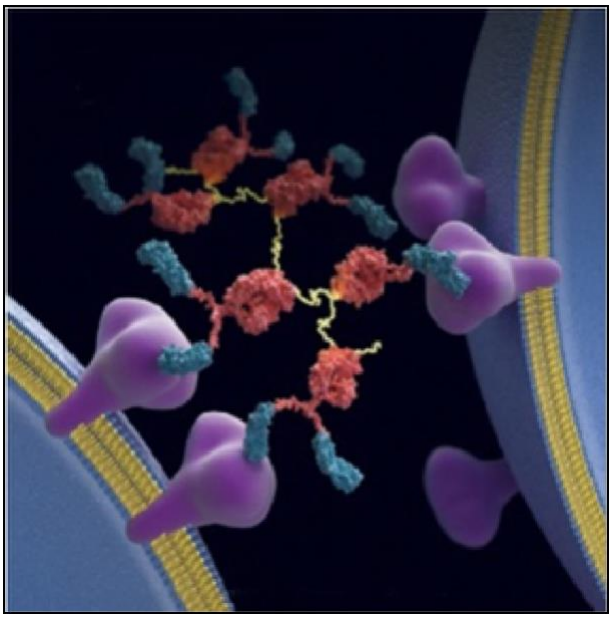


Figure 5: Recombinant hexeramized IgG consisting of 6 IgG1 subunits and three IgA tails holding it all together. The Fv region has been substituted with CD4 receptor making this a dodeceramized CD4 receptor that can cross-link HIV and inactivate it. When produced in CHO cells in flasks 40% of the protein is expressed as an improperly folded monomer, the exact same cells were seeded into the hollow fiber bioreactor and 95% of the protein is now expressed as a properly folded hexamer.

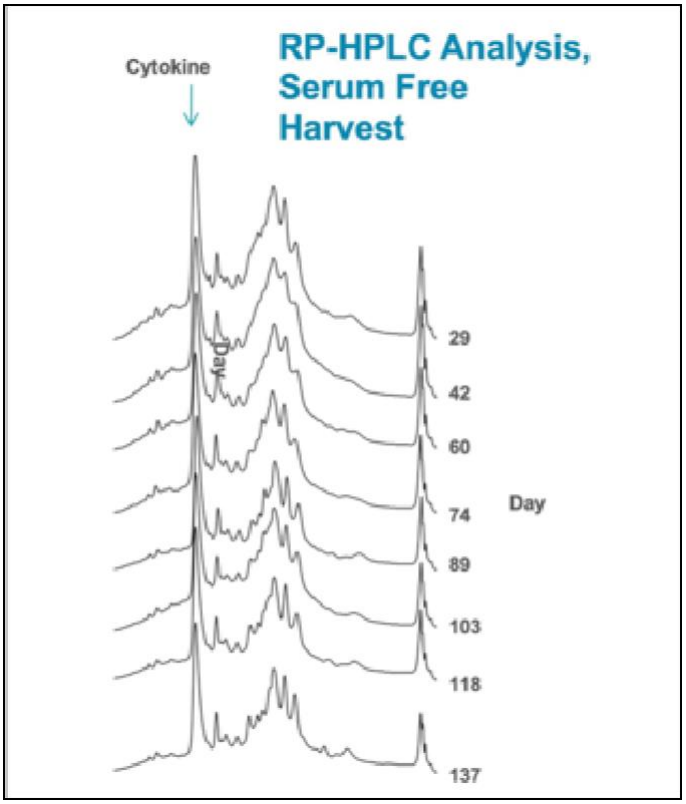


Figure 6: Il15 receptor complex expressed in 293 T cells demonstrating complete and uniform post translational modifications of this difficult to express protein over 140 days of continuous culture.

A) Physiologic Medium

Fetal bovine serum (FBS) has been a mainstay of cell culture despite its variability and potential for contamination. There are many shortcomings to the use of FBS: it is inherently variable, subject to geographic, seasonal, and inter-animal variations, prone to adventitious agents such as viruses and mycobacteria, and difficult to transport and store. Cells *in vivo* are exposed to interstitial fluid, not blood. The composition of the interstitial fluid is quite different from blood.

High-density cell culture allows cells to generate their own microenvironment, facilitating the use of chemically defined mediums such as FiberCell's CDM HD. A hollow fiber bioreactor recirculates freshly oxygenated medium from a larger central reservoir, providing homeostasis for nutrient delivery and waste removal, mimicking *in vivo* conditions.

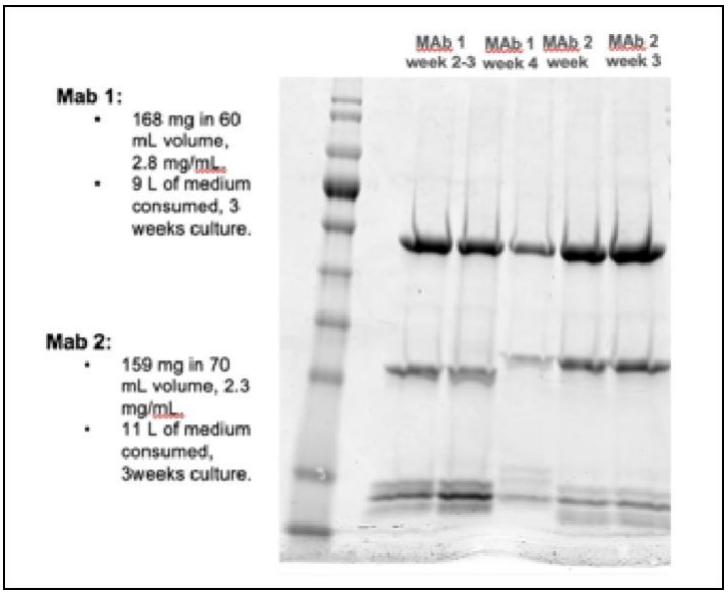


Figure 7: Monoclonal antibodies produced in the hollow fiber bioreactor using chemically defined CDM HD. These are unpurified supernatants and show the antibody of interest is the major component of the harvested supernatants.

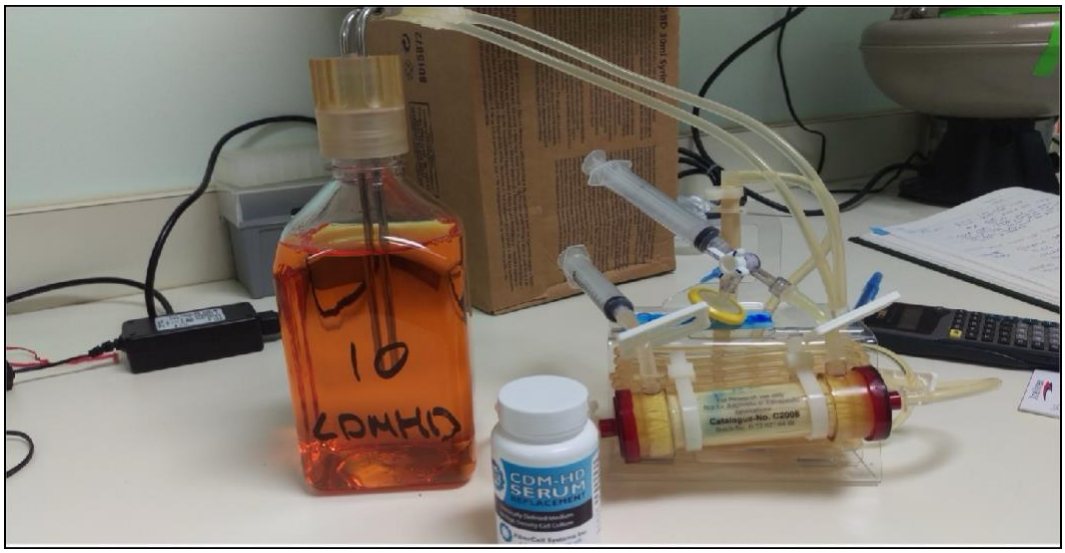


Figure 8: CDM HD is protein free, animal component free, chemically defined and cGMP compliant. It is simplified and optimized for the high cell densities of the hollow fiber bioreactor and allows the cells to generate their own microenvironment.

B) Physiologic Gas Levels

Cells within the body often experience much lower oxygen levels than the environmental norm. Traditional bioreactors and flasks have diffusional lags between headspace gas and dissolved gas. Hollow fiber bioreactors provide uniform and consistent gas exchange, with cells always within 100 µm of the fibers.

Figure 9: The hollow fiber bioreactor fits inside a standard CO2 incubator. Temperature and gas are controlled by the conditions of the incubator. Reduced handling time and no cell passaging reduces the time the cells are outside of the incubator.

C) Physiologically Relevant Structures for Cytocentric Conditions

3-D culture systems are inherently more cytocentric than 2-D cultures. The culture of cells in a monolayer is inherently non-physiologic. Cells *in vivo* never grow in this geometry. Cells must be periodically passaged or split to maintain the cells as a monolayer. In a hollow fiber bioreactor, the cells are attached to a porous surface, not a non-porous plastic dish. They are free to grow post-confluent and form 3-dimensional structures. High cell density promotes cell-to-cell interactions. Splitting is not required, and passage number is irrelevant. This is a huge advantage as the cell surface receptors are not digested and the cells don't need to reattach. Continuous culture without passaging maintains cell surface receptors and prevents reattachment issues in a cytocentric fashion. This is demonstrated by long-term hybridoma and glioma cell line cultures that were maintained for a year or longer of continuous culture.

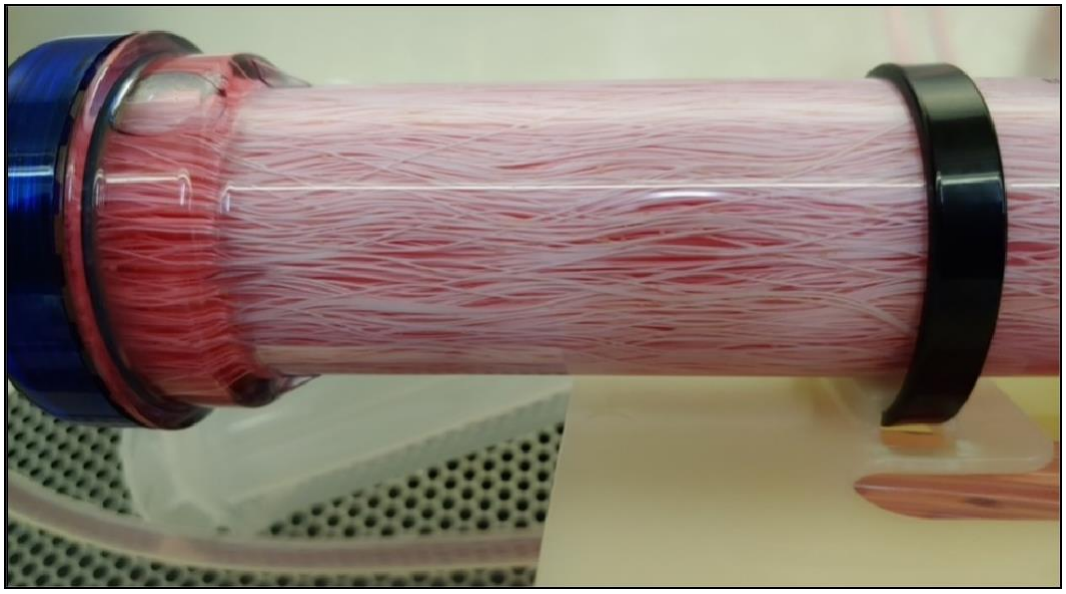


Figure 10: Porous support and continuous perfusion allows the cells to form 3-D structures such as the spheroids formed by mesenchymal stem cells seen here.

3. Cells Need Full-Time Optimal Conditions

Homeostasis is the key to cell physiology. The large medium reservoir and active recirculation ensure continuous consistency of nutrients and waste. Per protocol, the medium should be changed before there is any pH shift. The reduced handling time keeps the cells at optimal incubator conditions with fewer interruptions. This is cytocentric.

4. Cells Need Individualized Conditions

Individual cell types have differing requirements depending upon location, function, and physiology. The differences in micro-environment and effects on cell physiology are nuanced and incredibly complex. The cell-specific microenvironment must come from the cells themselves, along with active and passive control from the endothelial barrier. The cell- specific microenvironment is a dynamic interaction between the cells and the circulatory system. A truly cytocentric bioreactor promotes self-generation of the cell-specific microenvironment by the cells themselves. This is what occurs in a hollow fiber bioreactor. This is demonstrated by the near-universal application of CDM HD to many cell types.

Figure 11: Chemically defined CDM HD can support many different cell types provided the cells are at physiologic densities.

5. Cells Need Dynamic Environments as Populations Evolve

When there are large numbers of cells in a bioreactor, subpopulations of cells can proliferate at different rates, senesce, compete, and die. As subpopulations and total cell numbers grow and

change, their nutritional requirements are also likely to change. The relatively large medium reservoir of the hollow fiber bioreactor, which can be changed to respond to these conditions, is an integral part of the homeostatic physiologic support cells receive in the cytocentric hollow fiber bioreactor.

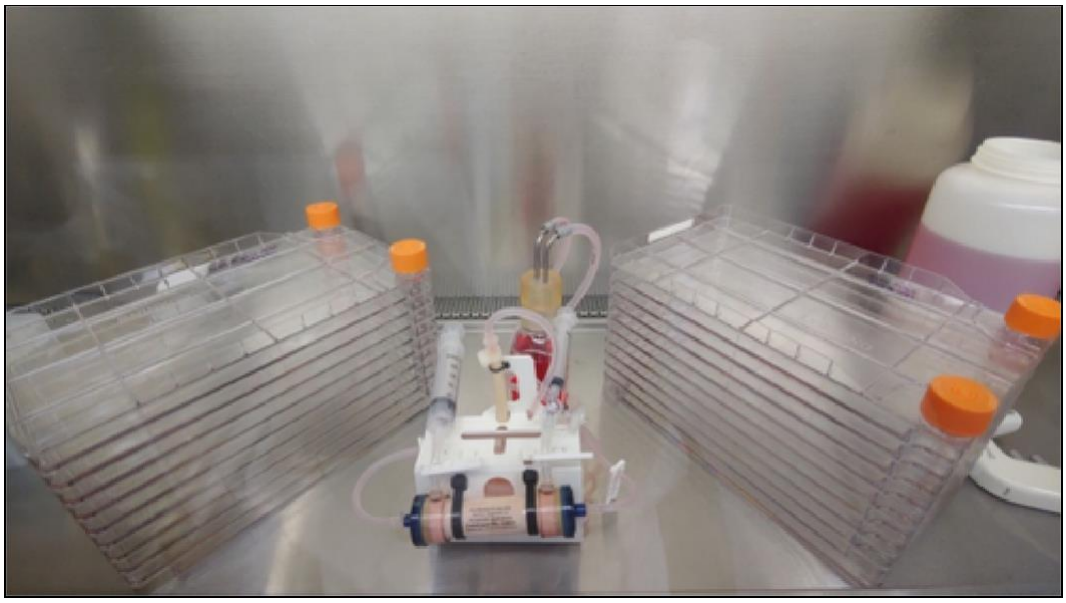


Figure 12: Large numbers of cells can be maintained for many weeks and months of continuous culture.

Cytocentric, Histocentric and Beyond

A cell-centric approach to cell culture and bioproduction recognizes the importance of external, controllable factors that produce reproducible results. There is a certain hubris to the expectation that ALL variables making up a complex cell micro-environment can be defined and controlled by external means. Acknowledging the cell's contribution to its own microenvironment, especially through autologous cytokine support, is crucial.

A 3-D perfusion artificial capillary hollow fiber bioreactor is the only bioreactor that can support cells at *in vivo* cell densities, 1-2 × 10^8 cells/ml. This high-density environment allows for simplified and optimized medium requirements, as demonstrated by CDM HD serum replacement. CDM HD is a near-universal, chemically defined serum replacement that supports various cell types at high densities, reflecting a concept beyond cytocentricity by also incorporating the cell's contributions to its micro-environment. This "histocentric" view recognizes the role the cells' own contributions play, as they do in tissues.

Histocentric Bioreactors

Histocentric bioreactors are designed to mimic the physiological conditions of tissues or organs in vitro, aiming to provide a more realistic environment for cell culture and tissue engineering. These bioreactors integrate several key principles

1. Biological Mimicry: Histocentric bioreactors aim to replicate the physiological conditions of the target tissue or organ as closely as possible. This involves recreating factors such as mechanical stresses (e.g., shear forces, compression), biochemical gradients (e.g., oxygen tension, nutrient supply), and spatial organization (e.g., cell-cell interactions, extracellular matrix deposition).

2. Controlled Environment: These bioreactors provide precise control over environmental parameters critical for cell growth and function. This includes control over temperature, pH, oxygen levels, nutrient concentrations, and waste removal.

3. Dynamic Culture: Many histocentric bioreactors incorporate dynamic conditions to simulate physiological movements or flows within tissues or organs. For example, they may apply mechanical stimuli such as fluid shear stress or cyclic stretching to mimic the mechanical environment experienced by cells *in vivo*.

4. Microenvironment: The bioreactors regulate the local microenvironment around cells or tissues. Cells cultured at high density can generate their own specific microenvironments. This involves gradients of signaling molecules, growth factors, and metabolites, which are crucial for cell differentiation, proliferation, and matrix production.

5. Compatibility with Cells: Histocentric bioreactors are designed to be compatible with various types of cells and tissues. Extra-cellular matrices can be exogenously applied or secreted by the cells themselves. They support the attachment, proliferation, differentiation, and function of primary cells, stem cells, or engineered cell lines, depending on the specific application.

6. Long Term Culture: A histocentric bioreactor does not require cell splitting, passaging or other disruption of the culture. Cell-to-cell interactions develop over time, and complex 3-D structures can be formed. Examples are bone marrow stroma cultured with hematopoietic stem cells or placenta co-culture resulting in MSC precursors being generated.

Overall, histocentric bioreactors represent a significant advancement in tissue engineering and regenerative medicine by providing a more physiologically relevant environment for cells and tissues outside the body. Their principles aim to bridge the gap between traditional cell culture techniques and the complex, dynamic conditions found in living organisms.

By adopting cytocentric and histocentric principles, researchers can achieve more reproducible and physiologic results, paving the way for advancements in regenerative medicine and cell therapy. A 3-D perfusion artificial capillary hollow fiber bioreactor adheres to the principles of a cytocentric and histocentric bioreactor. They support high cell density, active perfusion of nutrients and gas, and long-term continuous culture in an *in vivo*-like and cytocentric fashion. They go beyond "cytocentric," a cell-centric approach, to the next level up in organization, "histocentric", a tissue-centric approach. FiberCell Systems, a better way to grow cells.

References:

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2. Karnielli, et al. (2017). "A Consensus Introduction to Serum Replacements and Serum- Free Media for Cellular Therapies." Cytotherapy, 19(2), 155-169.
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