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Tech Note

Continuous Production of Exosomes

Utilizing the Technical Advantages of Hollow-Fiber Bioreactor Technology

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Interest in exosomes has grown of late, particularly since they have been seen to take part in functional interactions with antigen-presenting cells, and in the modulation of the immune response in vivo. For example, tumor exosomes are thought to participate in metastasis of tumor cells, seeding tumor-draining lymph nodes prior to tumor cell migration, and increasing their motility.

These nanovesicles have already proven themselves as having such therapeutic potential as the repair of cardiac tissue after heart attack. Clinical trials for many indications, such as using dendritic cell-derived exosomes to facilitate immune response to cancers, are now underway. As their content is a fingerprint of the type and status of the cell generating them, their prognostic potential as biomarkers, including biomarkers that could be used to predict cell therapy outcome, is being explored.

Current work on exosome manufacturing involves their regulatory-compliant generation in an appropriate cell line and separation from such process-related contaminants as other extracellular vesicles. In current practice, their production can be viewed as sequentially involving at-scale culture of the parent cell line; collection or harvest from the conditioned medium; and purification.

In lab-scale production, any number of culture, harvest, and purification approaches have been reported. Regulated manufacturing-scale production in classified environments, however, imposes constraints upon the production platforms considered. Cells currently employed in large-scale exosome production include mesenchymal stem cells, dendritic cells, and 293T cells.

Curent Production Platforms

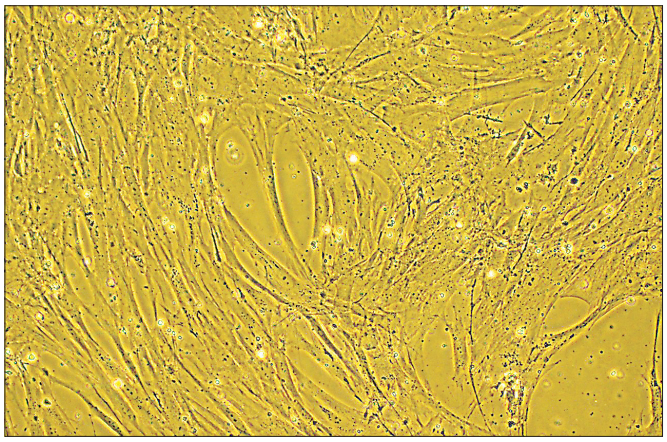
Current production platforms include scale-out in a large number of T-225 flasks, stacked array multiplate flasks, and packed-bed bioreactors. Exosomes are secreted in small quantities, and a standard batch-mode preparation can entail a final stage of hundreds of T225 flasks or rather expensive and technically challenging multiplate flask systems—with both yielding large volumes of medium to process.

In any of these approaches, it can take several rounds of splitting and culture expansion to attain this final production cell mass. They can also present such technical challenges as cells undergoing apoptosis just prior to harvest, contaminating it with membrane fragments.

Culture medium for manufacturing must not only support the robust expansion of the parent cell line, but also reduce process-related contaminants and support efficient exosome purification. An important issue here is that fetal bovine serum (FBS) contains a significant amount of endogenous exosomes that can contaminate the secreted exosome product. For production in classical media-based production (such as DMEM/10% FBS), this means eliminating serum following cell-mass expansion, or employing exosome-depleted animal serum. Some versions of this are now commercially available.

Serum-free medium (SFM) is an obvious option, but here it is important to insure the platform cell line expands robustly at scale in the production mode of choice, and that the SFM specifically supports the quality of exosomes desired.

After these 30–120 nm lipid microvesicles have been harvested or collected, purification (or fractionation) at lab-scale can be addressed by a variety



Human adipose-derived mesenchymal stem cells after adaptive passaging with HyClone™ AdvanceSTEM™ Medium and Serum in preparation for bioreactor seeding. Cells measured >99% positive for CD90-APC, CD73-PE, and CD105-PerCP. Julie Morris

of means. Methods described employ one or more distinct properties or characteristics of exosomes, including their size, density, morphology, composition, zeta potential, biochemical, or immunological features. Methods used include velocity and density gradients; agglomeration or precipitation via volume-excluding polymers (such as PEG or identified peptides); and adsorption chromatography (possibly by means of proprietary active-ligand-coated beads, which are found in a number of commercially available kits).

The need for economical and large-scale purification in a regulated environment, however, imposes limits on the technologies that can be considered. Currently popular large-scale methods include multiple rounds of centrifugation or filtration, followed by ultracentrifugation and density or velocity gradient centrifugation.

Scalable Exosome Production

Hollow-fiber bioreactors (HFBRs) have been employed in biotechnology for over 40 years, and their utility in the efficient and scalable production of exosomes has recently been reported. They provide extremely high surface-to-volume ratios, supporting large numbers of cells at high densities.

The molecular weight cutoff of the fiber, 5–20 kd, allows nutrients and waste products to pass through but retain such larger secreted product as proteins, antibodies, viruses, and exosomes, which are accumulated and concentrated by up to 100×. The cells are bound to a porous support so that splitting is not required, and many cell lines grow to post-confluence without significant apoptosis.

In some applications, collection of secreted products can be maintained over several months of continuous production. All of these factors combine to allow exosomes to be secreted in large numbers and concentrated significantly in the small volume of the extracapillary space of the cartridge. Importantly, exosomes cannot cross the fiber in either direction, so standard serum (such as HyClone™ Characterized FBS #SH30071, **GE Healthcare**) can be used in the circulating medium without contaminating the secreted exosomes within the extracapillary space of the cartridge.

Table 1. Exosome Production in Flasks vs. HFBR

		Duration (Days)	Volume (mL)	Protein (mg)	Exosomes (x109)	Medium Used (Total in L)
HFBR	Harvest 1	14 (medium)	40	1.87	22	7
	Harvest 2	14 (medium)	40	2.77	320	
	Harvest 3	14 (medium)	40	1.95	14	
	Harvest 4	14 (medium)	40	1.2	17	
	Total	56 (culture)	160	7.79	373	
Flasks	Harvest	02 (medium)	4,000	0.9	16	24

Adipose-derived MSC exosome production in flasks (T225) vs. HFBR (FiberCell C2011, 20 mL, 20 kd MWCO, PS cartridge). Flask cultures were expanded to 130 (total flasks) through sequential splitting 1:5 in DMEM/10% FBS. This was subsequently replaced with DMEM alone and harvested from all flasks 2 days later. One HFBR was seeded with 3X108 adipose derived MSCs in DMEM/10% FBS (in the intracapillary space, ICS). After culture equilibration, 40 mL extracapillary space harvests were performed every two weeks for 8 weeks. Total exosomes collected from one HFBR equaled that from (3032) T225 flasks.

hMSC-Based Exosome Production

Human adipose-derived mesenchymal stem cells were cultured in an HFBR using serum-containing medium (*Figure 1*). A C2011 cartridge (**FiberCell Systems**) was seeded with 3×10^8 MSCs obtained from adipose tissue (**Zen-Bio**). It continually produced exosomes for 10 weeks (*Table 1*). The typical harvest from the extracapillary space of the cartridge was 1.1×10^{12} exosomes/mL in 40 mL. Control cultures (25 × T225 flasks) yielded 1.4×10^{10} exosomes/mL in a volume of 400 mL.

The total bioreactor yield or exosomes by number was therefore approximately 10-fold higher at a concentration that was also 10-fold higher. Harvests were performed every two weeks, and prior to each harvest, small samples of cells were collected for phenotypic analysis.

During these 10 weeks, the culture did not expand based upon glucose uptake rate, which remained fairly constant. By orthogonal measure, the phenotype of the cells remained constant as well. Exosomes were continuously harvested without splitting and subculturing of the cells.

HFBRs have demonstrated potential in the manufacturing-scale production of exosomes using therapeutic entity production-grade materials. This method additionally provides a number of advantages to flask-based methods and has the potential for clinical-grade and large-scale manufacturing (*Table 2*). GEN

Table 2. Exosome Production Advantages

- Exosomes concentrated by a factor of 10–100X
- No serum-endogenous exosome interference (from ambient serum or serum-free replacement)
- Low contaminants and dilution eases purification
- Reduction in apoptosis can reduce cellular debris
- Typical serum starvation phenotype not observed
- Continuous production for over several months
- Passaging of primary/labile cells is not required
- Production system is scalable, single-use, closed
- High-density culture lowers production footprint
- Yields continuous biomanufacturing opportunity
- Scalable to even higher production in lager HFBR

Advantages of exosome production in SFM in a HFBR over standard serum containing media based production in T-flasks or related techniques.

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