Closed-System hMSC Working Cell Bank for use in Scalable Stem Cell Biomanufacturing

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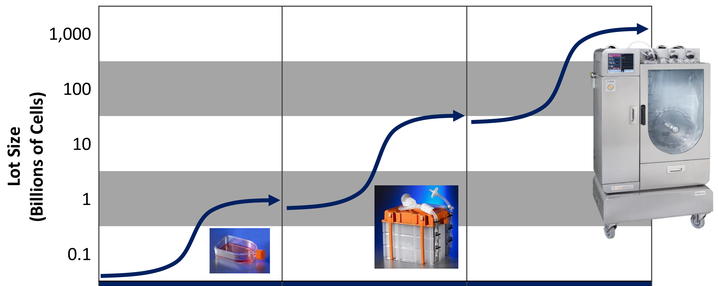
ABSTRACT

- Over 800 active clinical trials involve mesenchymal stem/stromal cells (MSCs), indicating an upcoming wave of demand for clinical-grade cells [Olsen et al, Frontiers in Medicine '18].
- There is a critical need for economical biomanufacturing processes capable of generating billions to trillions of cells per manufacturing lot to meet the demand of future commercial applications.
- A required component for realizing scalable platform systems capable of large scale hMSC production is the availability of a closed-system (CS) working cell bank (WCB).
- We have developed a scalable CS-WCB in a cryobag configuration containing xeno-free (XF) hMSCs, and established comparability for use in large scale closed system biomanufacturing.
- hMSCs cryopreserved in a CS storage format maintain comparability in hMSC critical quality attributes (trilineage differentiation potential and cell surface marker expression) as well as functional properties (angiogenic cytokine secretion and inducible immunomodulatory potential) when compared to the traditional cryovial storage format.

SHIFTS IN WCB CONFIGURATION WITH INCREASING SCALE



	Un-optimized T-Flask Production	Multi-Layer Vessel Production	Suspension Bioreactor Production
Production Lot Size	1B	30B	900B
Initial Cells Needed*	4M	120M	4B
WCB Format	1 x 5M CryoVial (1ml)	6 x 20M CryoVials (2ml each); 1 x 120M CryoBag (12ml)	200 x 20M CryoVials (2ml each); 1 x 4B CryoBag (400ml)

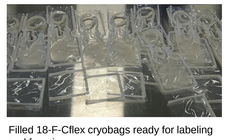
* Assuming 2 passage expansion

- hMSC manufacturing platforms are evolving as the demand for cells increases and the WCBs used during the production process need to scale proportionally.
- Traditional cryovial WCB configurations for seeding T-flasks or Multi-Layer Vessels are manageable for production lot sizes of < 30B cells, however, cryovials become impractical for seeding a bioreactor platform where the number of cells needed for seeding increases markedly and closed system processes are preferred due to their lower inherent risks.
- Cryobags allow more flexibility in fill volumes while reducing the amount of labor and time needed to prepare and thaw cells.

CRYOBAG SELECTION

CryoBag	Animal Derived Component Free (ADCF)	C-Flex Welding Compatibility	Bag Integrity/Durability*
Saint Gobain (18-F-CFLEX)	✓	✓	✓
Bag 2		✓	✓
Bag 3		✓	✓
Bag 4			✓
Bag 5			✓
Bag 6			✓

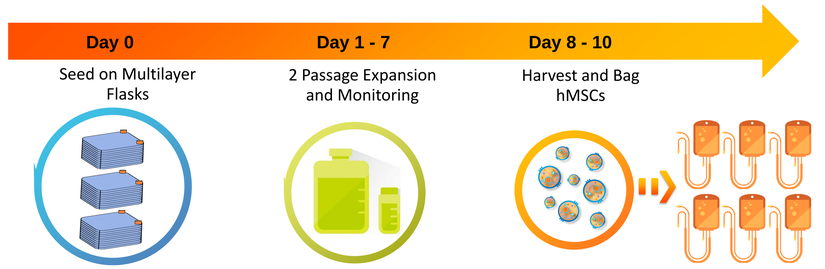
* Bag integrity and durability was determined by freeze/thaw cycling and a 6-sided drop test from a height of 3'.



Filled 18-F-Cflex cryobags ready for labeling and freezing.

- The Saint Gobain 18-F-CFLEX bag and tubing are completely ADCF and the bag film remains flexible at LN2 temperatures (vapor phase) unlike the other bags tested. Additionally, the C-Flex tubing is protected and enclosed inside of its own sterile pouch.

MANUFACTURING PROCESS

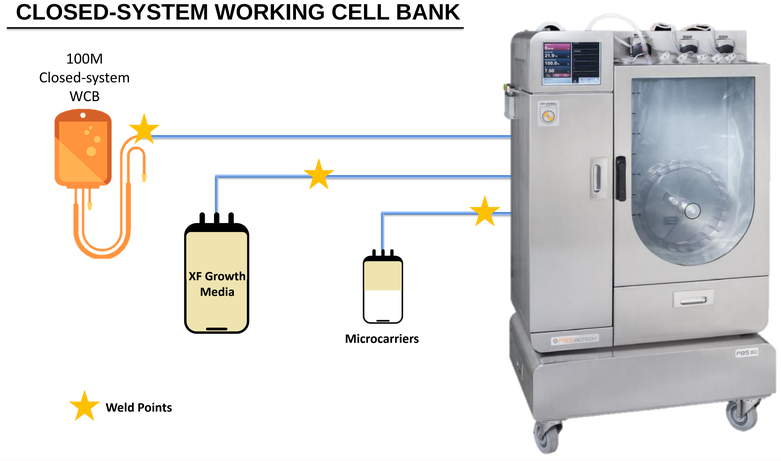


- Two half scale and one full scale manufacturing runs were completed using XF hMSCs from three distinct donors.
- Once thawed, the closed-system WCB is designed to be weldable to existing bioreactor lines reducing process risk, time, and labor (image below not to scale).

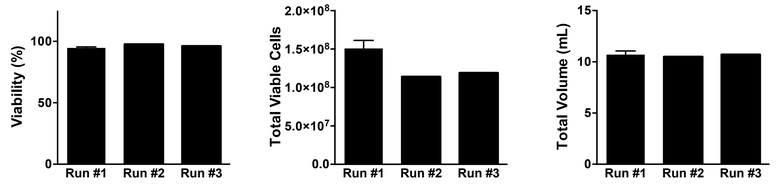
Run #	Scale	Donor #	Total Bags Filled
1	Half	1	18
2	Half	2	28
3	Full	3	40*

* A total of 60 bags could have been filled but run was limited by # of cryobags available.

CLOSED-SYSTEM WORKING CELL BANK



CONSISTENT PRODUCTION

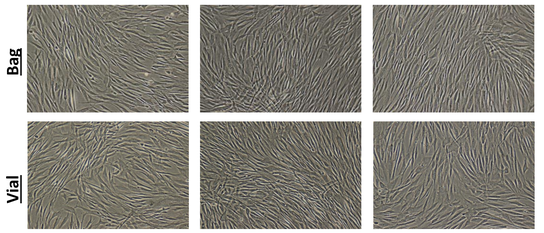


- Cryobags were thawed using a VIA Thaw CB1000 automated bag thawing device and the contents removed via a syringe adapter.
- Fill volumes and total viable cell counts were consistently ≥10ml and ≥100M cells, respectively with post thaw viability maintained above 80% for all runs meeting the target product specifications.

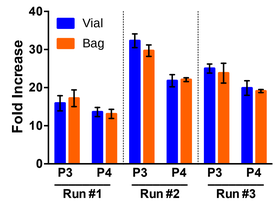
TARGET PRODUCT SPECIFICATIONS		Criteria Met
Product Name	RoosterBank™-hBM-100M-XF	
Part #	MSC-035	
Product Description	Closed-system working cell bank in a cryobag configuration with a weldable (Cflex) tube port, with a 10ml fill consisting of ≥100M viable cells.	
Recovery: Cell #	≥100M viable cells	✓
Recovery: Viability	>80%	✓
Expected Launch Date	Q1 2019	

COMPARABILITY OF CELLS: CS-BAGS vs VIALS

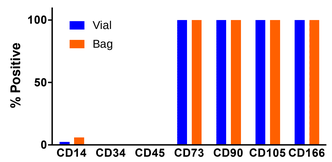
(A) MORPHOLOGY



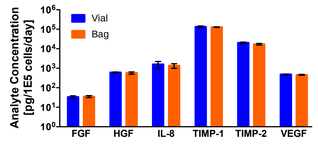
(B) EXPANSION



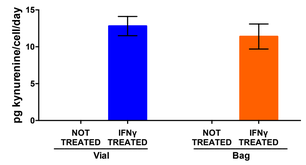
(C) SURFACE PROTEIN EXPRESSION



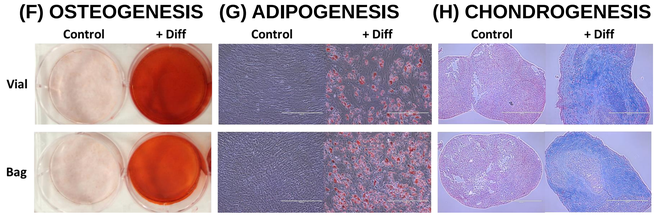
(D) CYTOKINE SECRETION



(E) IDO ACTIVITY



(F) OSTEOGENESIS (G) ADIPOGENESIS (H) CHONDROGENESIS



- XF hMSCs generated from the CS WCBs maintained typical phenotypic properties, including (A) MSC morphology; (C) classic surface protein expression; and tri-lineage differentiation potential to (F) osteogenic, (G) adipogenic, and (H) chondrogenic lineages when compared to cryovial storage.
- (B) Expansion potential and functional properties were also maintained with comparable levels of (D) secreted angiogenic cytokines and (E) inducible indoleamine 2,3-dioxygenase (IDO) activity when stimulated with interferon-gamma (IFN_γ).
- A stability protocol will be executed to determine changes in these properties over time and to establish a shelf-life for the product (below).

STABILITY TESTING FOR SHELF-LIFE

Timepoint (Months

Test	Release	6	12	18	24	30
Sterility	✓			✓		
Mycoplasma	✓					
Recovery: Cell Number	✓	✓	✓	✓	✓	✓
Recovery: Viability	✓	✓	✓	✓	✓	✓
Performance Test (FIO)	✓	✓	✓	✓	✓	✓
Differentiation (FIO)	✓	✓	✓	✓	✓	✓
IDO/Cytokine (FIO)	✓	✓	✓	✓	✓	✓
Flow cytometry (FIO)	✓	✓	✓	✓	✓	✓

CONCLUSIONS

- A closed system working cell bank was developed for use in scale up hMSC manufacturing and demonstrated comparable post thaw cell health, function, and potency to the traditional cryovial format.
- A total of 3 manufacturing runs were executed yielding a total of 86 100M XF hMSC closed system WCBs that can be utilized in scale up bioreactor expansion of hMSCs.

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