Scalability of Allogeneic Stem Cell Manufacturing

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Introduction

Stemedica Cell Technologies manufactures in-house clinical GMP grade non-embryonic stem cells, including bone marrow derived human mesenchymal stem cells (hMSCs) and human neural stem cells (hNSCs). Patients are currently being enrolled in US human clinical Phase 2a trials using hMSCs to treat stroke, acute myocardial infarction, chronic heart failure and skin photoaging.

In anticipation of dose demands for Phase 3 clinical trials, a scale-up process for the manufacture of hMSCs is being developed. The challenge is to identify scalable, robust and cost-effective upstream and downstream platforms. In addition, the clinical delivery dose preparation must be user friendly and preferably off-the-shelf. Current developmental efforts to scale and optimize hMSC production and dose preparation are presented here.

Evaluating Scale-Up Cell Expansion Platforms

- New upstream scale-up platforms are available that offer a combination of a larger growth surface, smaller footprint and control of culture environment.
- Challenges exist for the implementation of each platform (Figure 1) and criteria needs to be established to ensure the most suitable platform is chosen (Figure 2).

FIGURE 1

Key challenges to scaling up.

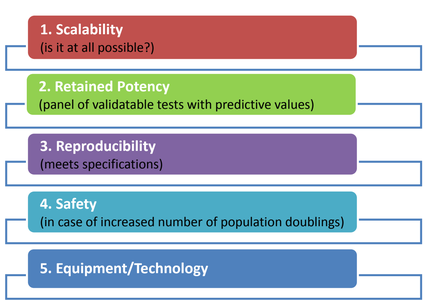
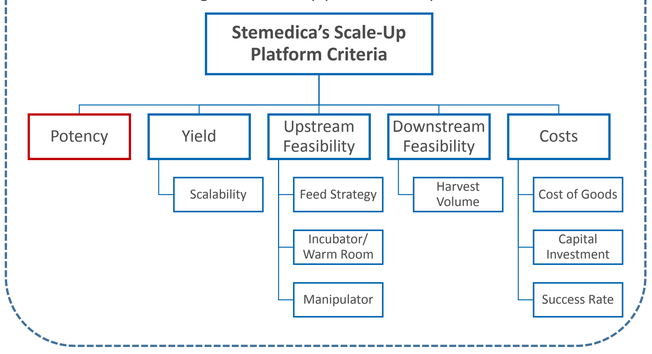


FIGURE 2

Criteria for determining which scale-up platform to implement.



Stemedica's Scale-Up Process

Stage 1

- From tissue culture dishes and triple layer flasks to 1 and 5 layer CellStacks

Stage 2

- From 1 and 5 layer CellStacks to ???

Potential Scale-Up Platforms

Two-Dimensional (2D)

- 40 Layer CellStacks or Cell Factories
- HyperStacks (Corning)
- CellCube (Corning)
- Xpansion Bioreactor (Pall)

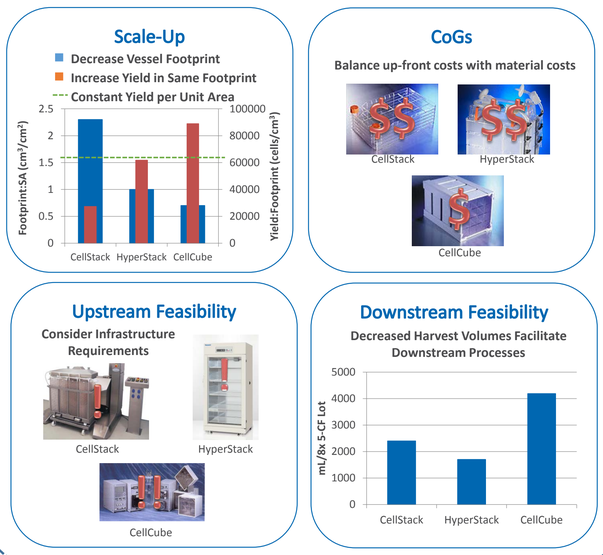
Three-Dimensional (3D)

- Microcarriers
- Packed Bed Bioreactor
- Hollow Fiber Bioreactor

➢ **Based on the scale-up criteria, Stemedica evaluated 2D platforms (Figure 3).**

FIGURE 3

2D Scale-Up Platform Comparison



hMSCs Retain Potency and Yield with HyperStacks

Yield and Viability

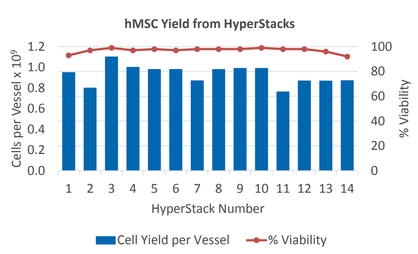
- hMSC growth kinetics in the HyperStack are similar to those in CellStacks.
- Optimization of seed density and/or culture are still necessary.

Identity and Potency

- hMSC expansions in HyperStack were tested for identity and potency.
- QC testing has demonstrated comparability to hMSCs expanded in CellStacks.

FIGURE 4

Vessel-to-vessel hMSC yields and viability from HyperStack expansions.



Optimization of Cryopreservative DMSO Content

The optimal cryopreservative DMSO content for Stemedica's hMSCs is being evaluated based on three criteria:

1. Must support cell quality during extended in-process hold times (cryopreservative addition to freeze start).
2. Must support cell quality during extended prepared dose storage times (preparation to patient dosing time).
3. To minimize the concentration of DMSO in the prepared dose administered to patient .

Post-thaw recovery with reduced DMSO

Method

- hMSCs were resuspended in cryopreservative containing either 2%, 5% or 10% DMSO, CryoStor CS2, CS5 or CS10 (BioLife Solutions, Inc., Bothell, WA), respectively.
- Cells were held at room temperature for two hours prior to being frozen in a controlled rate freezer.
- Cells were thawed and diluted in infusion solution prior to evaluating yield and viability using a hemocytometer.

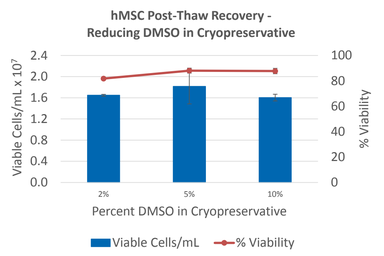
Result

➢ Cryopreservative containing 5% or 10% DMSO (CryoStor CS5 or CS10) supports post-thaw hMSC recovery (Figure 6).

➢ QC testing shows maintenance of identity and potency for hMSCs frozen in cryopreservative containing 5% or 10% DMSO (data not shown).

FIGURE 6

Lowering DMSO concentration in the cryopreservative to 2% leads to a reduction in cell viability.



Post-thaw recovery with extended in-process hold times

Method

- hMSCs were resuspended in cryopreservative containing either 5% or 10% DMSO, CryoStor CS5 or CS10, respectively.
- Cells were immediately frozen, or held at room temperature for two or five hours prior to being frozen in a controlled rate freezer.
- Cells were thawed and diluted in infusion solution prior to evaluating yield and viability using a hemocytometer.

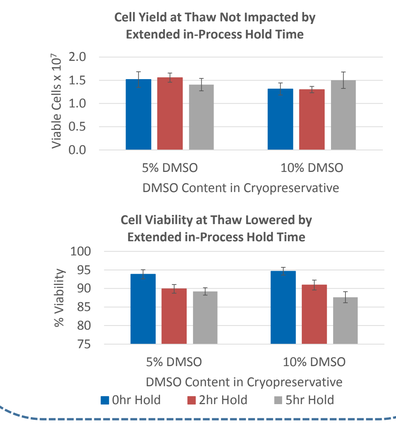
Results

➢ Longer in-process hold times may lead to reduced cell viability (Figure 7).

➢ Cryopreservative containing 5% DMSO (CryoStor CS5) maintains hMSC viability during a hold time of between 2 and 5 hours (Figure 7).

FIGURE 7

Cryopreservative with a lower concentration of DMSO may better accommodate a longer in-process hold time.



Cell characterization after prepared dose storage

Method

- hMSCs were resuspended in cryopreservative containing either 5% or 10% DMSO, CryoStor CS5 or CS10, respectively.
- Cells were held at room temperature for two hours prior to being frozen in a controlled rate freezer.
- Cells were thawed and diluted in infusion solution prior to evaluating yield and viability using a hemocytometer and/or Annexin V/Propidium Iodide staining.
- The prepared dose was stored for 6-8 hours and re-evaluated.

Results

➢ hMSC yield and viability is maintained during the prepared dose storage time when the dose contains 0.5% DMSO (Figure 8).

➢ Cell growth is not affected by the new dose preparation method (Figure 9).

➢ Annexin V/Propidium Iodide staining shows similar viability as Trypan Blue staining (Figure 8 and 9).

➢ Apoptotic cells die during the storage time (Figure 9).

FIGURE 8

Cell yield and viability is not affected by the new dose preparation and a dose hold time of up to 6 hours.

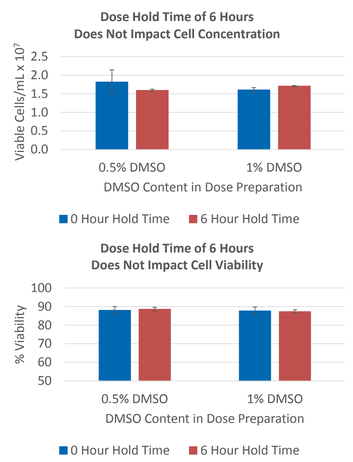
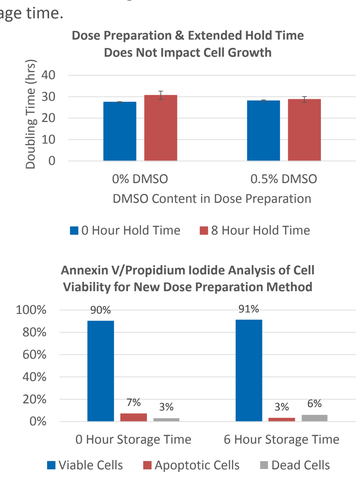


FIGURE 9

Cell growth and viability is not affected by the new dose preparation containing 0.5% DMSO and an extended dose storage time.



Cryopreservative with 5% or 10% DMSO supports hMSC Recovery

- **hMSCs frozen in CryoStor CS5 or CS10 have efficient post-thaw recovery and pass QC testing for identity and potency.**
- **CryoStor CS5 or CS10 supports hMSC viability during extended in-process hold times and prepared dose storage times.**

Improved Clinical Delivery Method

- Administration of cells to patients can require specialized clinical facilities and training.
- Aseptic Technologies' Crystal Vial Filling Station allows closed vials to be filled aseptically through a stopper using a dosing pump. The vial stopper is laser sealed post-fill to maintain sterility.

Current State	Future State
1.8 mL Cryovials	6 mL Crystal Vials
Open System	Closed System
Scale-Out (more vials)	Scalable (Up and Out)
Manual Fill	Manual or Automated Fill
~12 Vials Pooled per Dose	≤ 3 Vials Prepared per Dose
Cell Count at Clinic	Vial Filled with Required Dose
Formulation Required	Compounded Sterile Preparation
High Aseptic Risk at Clinic	Minimal Aseptic Risk at Clinic

FIGURE 5

Weight checks confirm a consistent fill volume.

Average of 7 Runs:	10.356 g
St. Dev. of 7 Runs:	0.021 g

Next Steps

Downstream Processing

As yields increase so do harvest volumes and therefore processing of the larger volume must be considered. Centrifugation of larger volumes adds a significant amount of processing time but is also an open process. Tangential flow filtration for the separation and concentration of cells is being explored for the future scale-up process. Closed system designs and automation are essential.

Microcarriers

The potential of microcarriers has been recognized and is also being explored. Microcarriers have been proven successful for the production of biomolecules, however much development is still necessary to identify the optimal culture and harvest conditions as well as to demonstrate product comparability.

Summary

Criteria for choosing a scale-up platform was discussed. Optimization and demonstrating comparability is essential to the success of scaling-up the manufacturing process.

The Crystal vial fill-finish system is being integrated into the hMSC production process to simplify dose preparation in the clinic. Moreover, the closed system can be automated to accommodate the scale-up large fill volumes.

Efficient post-thaw recovery of hMSCs in CryoStor CS5 or CS10 has been observed for extended in-process hold times and prepared dose storage times.

Acknowledgments

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