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Technical Note

Stemline® XF MSC Medium has High Yield and Functionality in the 3 L Mobius® Stirred Tank Bioreactor

Abstract

Optimizing *ex vivo* cell expansion processes in preparation for clinical use is a critical step in cell therapy manufacturing. Given the curative and lifesaving impacts these therapies can have on patients, performance and quality are essential outputs of any expansion process. Here, we discuss the performance of Stemline® XF MSC Medium, which promotes expansion of human mesenchymal stromal/stem cells (hMSCs) to high densities while maintaining cell identity and quality. This product was designed for efficient expansion in planar and microcarrier-based culture platforms, easing the transfer between research, clinical, and manufacturing scale culture. We describe the *ex vivo* expansion of bone marrow-derived hMSCs cultured in Stemline® XF MSC Medium in a 3 L stirred tank bioreactor-microcarrier platform.

Introduction

The long-term outlook for stem cell therapy predicts a shift toward more defined media and high-quality raw materials. Currently, fetal bovine serum (FBS) is still used in the majority of MSC products being developed for clinical use. For many reasons, cell therapy developers are being encouraged to move away from the use of serum at earlier stages of product development[1]. Alternative formulations include solutions often categorized as “serum free,” “xeno free,” or “defined.”

Developing a well-defined, scalable bioprocess is also important to produce robust and safe cell therapies[2]. To support that vision, there is an increased need for cell therapy reagents compatible with microcarrier-based suspension culture platforms. This would allow for both small-scale studies and large-scale therapeutic manufacturing, without the need to redefine critical reagents when entering a new phase of the therapy development process.

Pre-clinical and clinical level data will be essential in determining the safety and therapeutic effects for different disease indications for hMSC therapies[3]. Thus, it is extremely beneficial to investigate serum-alternative media formulations that support high performance expansion and are compatible with scalable manufacturing processes. Here we detail the process parameters and growth results of bone marrow-derived hMSCs cultured in Stemline® XF MSC Medium in a 3 L stirred tank bioreactor-microcarrier platform.

Methods

Stemline® XF MSC Medium, consisting of Stemline® XF MSC Basal Medium (**Cat. No. 14371C**) and Stemline® XF MSC Supplement (**Cat. No. 14372C**), was used in our 3 L Mobius® bioreactor system with minimal process modifications.

Bone marrow-derived hMSCs were scaled up in either Stemline® XF MSC medium, alpha-MEM supplemented with 5% human platelet lysate (hPL), or a commercially available xeno-free competitor medium. Each medium was supplemented with L-glutamine at a final concentration of 2 mM. Shear protectants were added to each medium. Collagen Type I-coated microcarriers were chosen for hMSC expansion.

Over the duration of the run, dissolved oxygen (DO) and pH were continuously monitored and controlled at 50% and 7.4 respectively. Nutrient and metabolite levels were measured daily (BioProfile® FLEX2, Nova Biomedical). If the L-glutamine or glucose levels fell below half the recommended concentration, they were spiked back up to the recommended level. Total cells were determined by manual sampling and counted in triplicate (Technical note No. 0221 Rev.1.2, Chemometec).

The hMSCs were harvested on day 8 of the bioreactor culture. Expression of the surface markers CD44, CD73, CD90, CD105, CD11b, CD14, CD19, CD34, CD45, CD79a, CD106, CD274 and HLA-DR was assessed by flow cytometry. Potency of the cells expanded in the Stemline® XF MSC medium was also determined. After bioreactor expansion, the hMSCs were re-plated in tissue culture flasks and activated (licensed) by supplementing the media with 25 ng/mL (final) of tumor necrosis factor (TNF)-α and interferon (IFN)-γ. After three days, the expression of the immune potency-related surface

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markers CD274, CD54, HLA-DR, CD80, CD40, and CD86 was determined by flow cytometry. The indoleamine 2,3-dioxygenase (IDO) activity of the activated hMSCs was also assessed by measuring the concentrations of L-tryptophan and L-kynurenine in the cell culture supernatant. Trilineage differentiation capability was tested using AdipoMAX Differentiation Medium (**Cat. No. SCM122-1KT**), ChondroMAX Differentiation Medium (**Cat. No. SCM123**) and OsteoMAX-XF Differentiation Medium (**Cat. No. SCM121**).

Summary: Run Parameters	
Cell Bank	Bone marrow-derived hMSC bank
Process/volume	2.4 L fed-batch (1 L + 1.4 L)
Duration	8 days
Seed density	3 x 10^3 cells/cm^2
Microcarriers	Collagen-coated polystyrene, 15 g/L (360 cm^2/g)
Media	Stemline® XF MSC medium Alpha-MEM + 5% hPL Xeno-free competitor
Temperature	37 °C
pH	7.4 (0.05 dead band) via CO_2/base
DO	50% via air/O_2/N_2 overlay
Agitation rate	35 rpm (day 0 – feed), 61 rpm onwards

Results

Overall, hMSCs expanded in the Stemline® XF MSC medium showed superior growth performance (**Figure 1**). After an eight-day culture, a yield of 8.3 E+08 total viable cells was attained with the Stemline® XF MSC medium, followed by 5.25 E+08 cells with AMEM + hPL, and 3 E+08 with the xeno-free competitor medium. The cumulative population doublings were 5.6 with the Stemline® XF MSC medium, 5.0 with AMEM + hPL, and 4.2 with the competitor xeno-free medium.

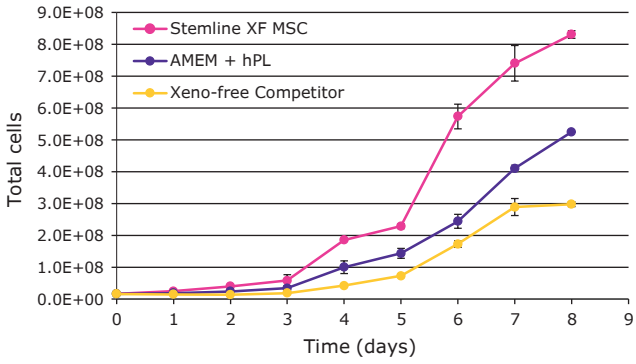


Figure 1. Comparison of growth on microcarriers in the Mobius 3 L bioreactor. Total cell counts on Day 8 indicate Stemline® XF MSC medium outperformed AMEM + hPL by 58.3% and a commercially available xeno-free formulation by 178.9%.

pH and DO remained within control constraints with notable spikes on Day 3 during addition of media and microcarriers (data not shown). Nutrient depletion and waste production in Stemline® XF MSC medium were increased compared to other media and are consistent with the higher growth rates (**Figure 2**).

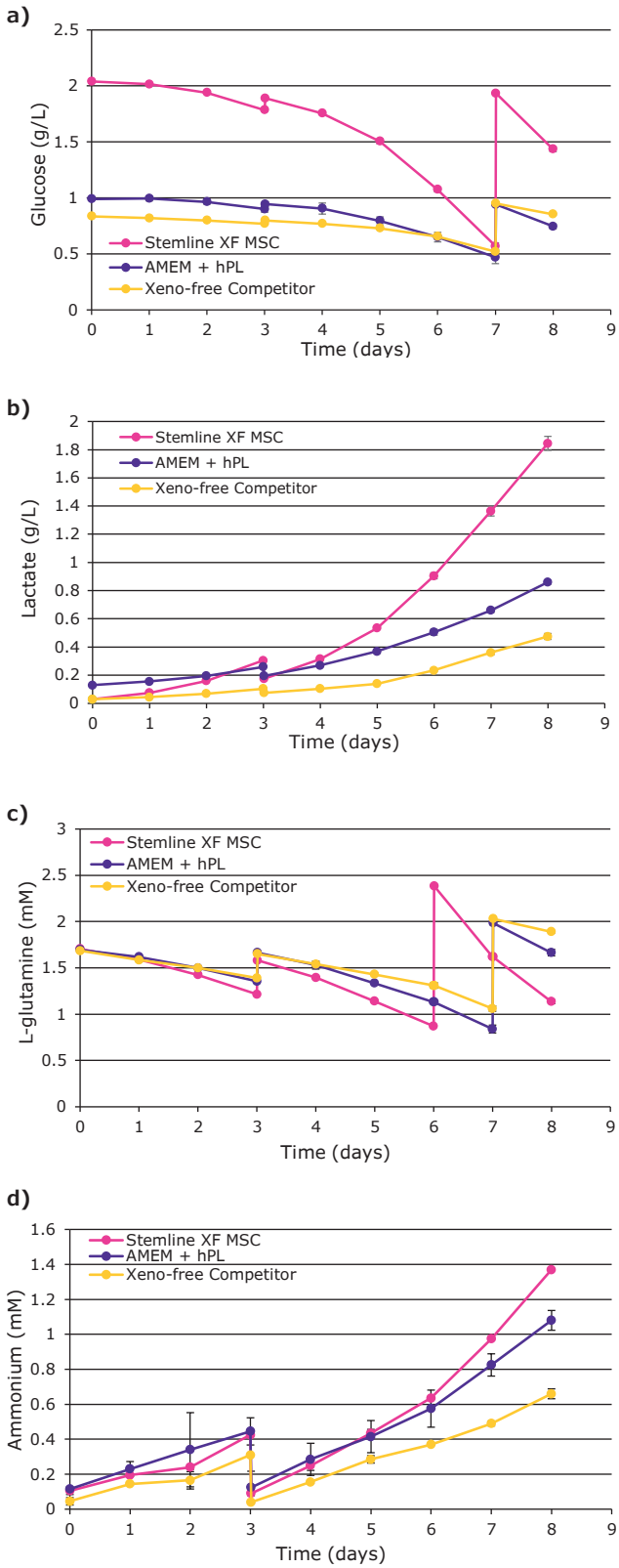


Figure 2 (A-D). Comparison of nutrient and metabolite profiles during expansion in the 3 L bioreactor. The hMSCs consumed more glucose (A) and produced greater levels of lactate (B) with the Stemline® XF MSC medium. Similarly, there was increased glutamine consumption (C) and ammonium production (D) with Stemline®.

We found that hMSCs expanded in Stemline® XF MSC Medium retained typical identity markers and potency after 3 L bioreactor culture. The cells highly expressed CD44, CD73, CD90, and CD105, concomitant with little to no expression of CD11b, CD14, CD19, CD34, CD45, CD79a, CD106, CD274 and HLA-DR (**Figure 3**). The hMSCs successfully differentiated into adipocytes, chondroblasts, and osteoblasts as shown by positive stains of lipid vacuoles with Oil Red O, glycoconjugates with Alcian Blue, and calcium deposits with Alizarin Red respectively (**Figure 4**). Several MSC surface markers related to immune function including CD274, CD54, and HLA-DR were upregulated after a three-day incubation with TNF-α and IFN-γ (**Figure 5**)[4]. There was increased IDO activity with licensed hMSCs which was confirmed through the significant depletion of L-tryptophan and production of L-kynurenine (**Figure 6**).

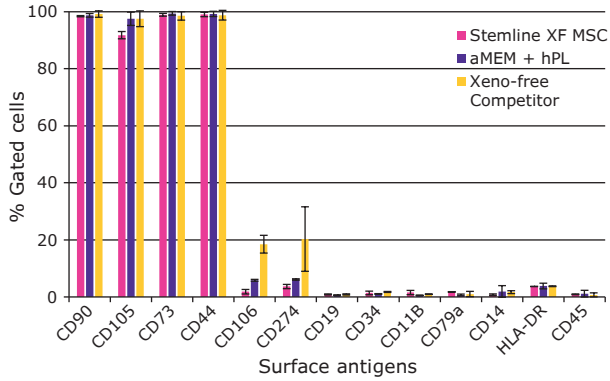
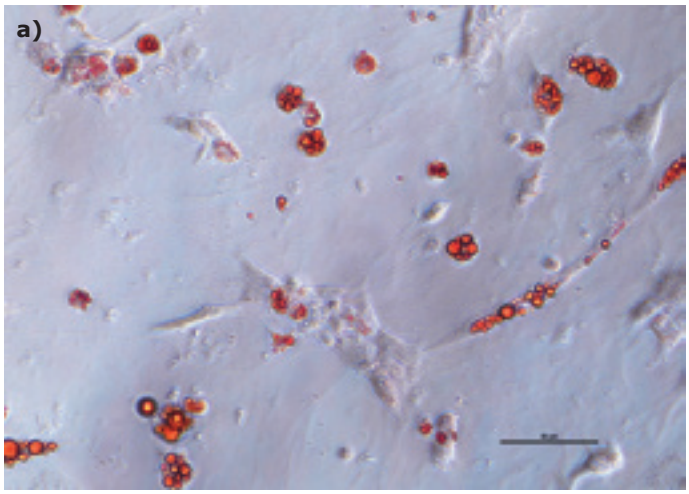
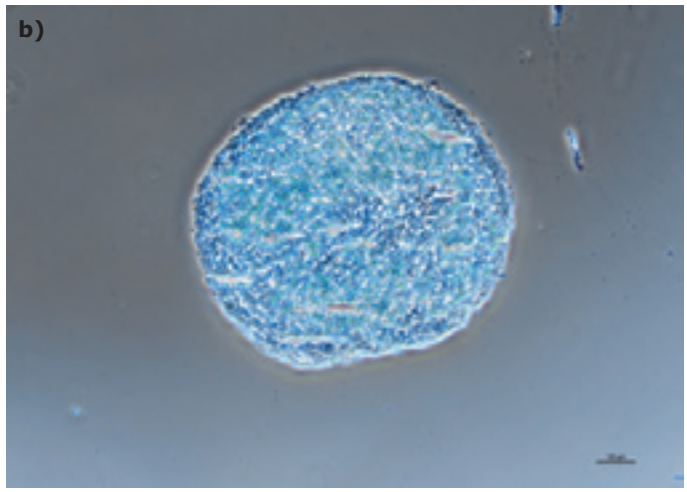


Figure 3: Comparison of surface marker expression post-expansion in the 3 L bioreactor. The typical bone marrow-derived hMSC surface marker phenotype was observed with all three media. There was positive expression of CD90, CD105, CD73, and CD44, along with negative expression of CD106, CD274, CD19, CD34, CD11B, CD79a, CD14, CD45, and HLA-DR.





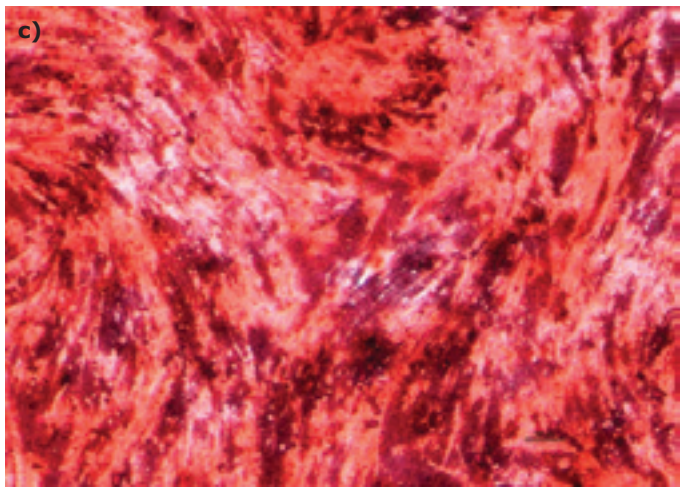


Figure 4: Trilineage differentiation of hMSCs after 3 L bioreactor expansion with Stemline® XF MSC medium. The cells maintained the ability to differentiate into adipocytes (A), chondroblasts (B), and osteoblasts (C) with positive tissue stains after respective differentiation procedures.

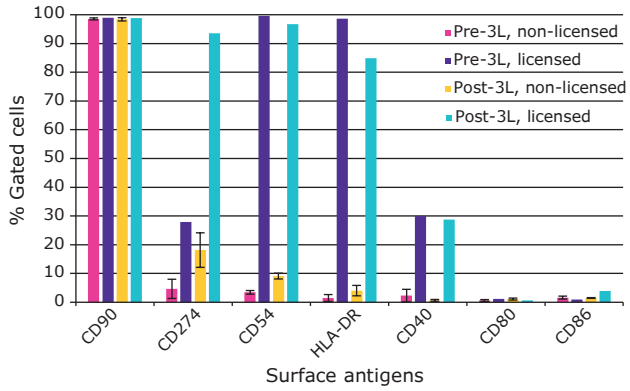


Figure 5: Upregulation of immunomodulatory surface antigens of licensed hMSCs cultured with the Stemline® XF MSC Medium. The cells retained the characteristic ability to modulate immune-related surface antigen levels after expansion in the 3 L bioreactor. There was increased expression of CD274, CD54, HLA-DR, and CD40. The marker CD90 was included as a control.

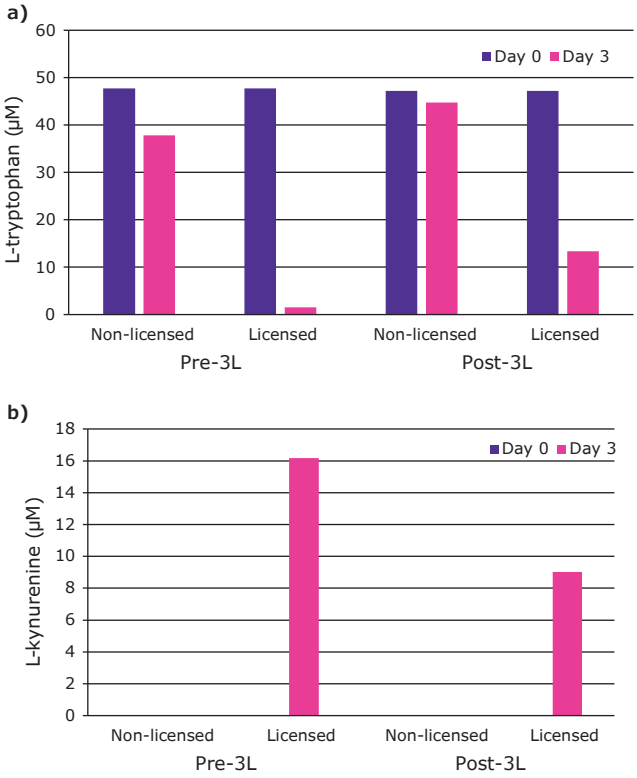


Figure 6. Human MSCs cultured with Stemline® XF MSC medium retain immunomodulatory capacity via IDO activity after expansion in the 3 L bioreactor. L-tryptophan was depleted in the media at an enhanced rate (A) and L-kynurenine was produced (B) with licensed cells.

Discussion/Summary

The Stemline® XF MSC medium consistently outperformed the other xeno-free media formulations tested in total cell yield with 3 L bioreactor expansion. The increase in hMSC growth rate in this media has obvious implications for manufacturing strategy, potentially allowing new products to have shorter expansion timelines and increasing savings on reagents, materials, and labor. The cells expanded in this medium also retained prototypical MSC identity markers and immunomodulatory capabilities after 3 L bioreactor culture.

Our data demonstrates the utility of Stemline® XF MSC Medium for bench-scale bioreactor expansion of hMSCs in microcarrier-based cultures. High yields of functional hMSCs were generated when compared with other xeno-free alternatives. High quality media and supplements are positioned to dynamically impact cell manufacturing processes and will enhance the development of the burgeoning cell therapy field.

References

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