Serum Alternatives for Adult Stem Cell Expansion for Allogeneic Therapy

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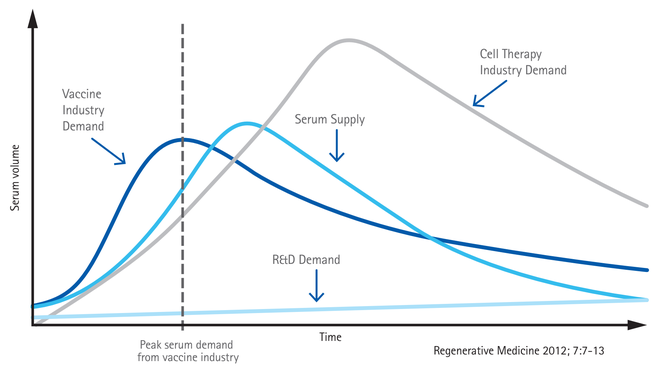
Introduction

As more stem cell therapeutics progress through clinical testing, current *in vitro* culture methods are proving cumbersome to scale. The combination of decreased demands for serum from the recombinant protein and vaccines markets and the long term view of regenerative medicine therapies predict a shortage of serum as clinical programs are successful. In addition, there is a desire to remove animal-derived materials from the production process to minimize risk to the patient for a product that is minimally processed.

Fetal Bovine Serum (FBS) supply at risk for cellular therapies

- 90% of serum currently utilized in commercial therapeutic manufacturing is sourced from Australia, New Zealand or the US
- A limited number of serum suppliers utilize facilities that meet ISO standards
- Much of the infrastructure required to provide high-quality serum has been scaled-back due to the decreased demand by the vaccine industry
- The predicted demand of the cellular therapy industry will not be met by the downward-trending serum supply



Experimental Approach

- Human platelet lysate (PL) was evaluated as an alternative supplement to bovine serum, including EMD Millipore's PLTMax human platelet lysate (Cat. No. SCM141)
- Multiple sources of human platelet lysate were tested:

Sample	Heparin
PLTMax hPL	+
B	+
C	-

- Mesenchymal stromal/stem cells (hMSCs) derived from human, adult bone marrow were used as a model cell type
- 3L bioreactors studies were included as more advanced suspension cultures, with cells attached to microcarriers
- Performance was assessed by monitoring growth, gene expression, cell surface marker expression and functional potential after expansion

Materials and Methods

hMSC culture in benchtop bioreactors

hMSCs were derived from adult bone marrow and banked at passage two. Mobius® CellReady 3L bioreactors with Finesse controllers were seeded with 5 x 10^6 hMSCs in 1 L of culture medium. 15 g/L of collagen-coated microcarriers were added to provide a surface area of 5,400 cm^2. Bioreactors were run for up to 14 days with a 5% CO_2 overlay. On day 6 bioreactors were fed with 1 L of respective FBS or PL-containing low glucose medium, and on day 9 they were fed with 0.4 L of high glucose medium. Cultures were agitated at 50 rpm in the beginning and with respective feeds the agitation speed was increased to 90 rpm and 100 rpm. Cell growth was monitored by NucleoCounter®. A BioProfile® analyzer was used for evaluation of glucose, lactate and ammonia levels during bioreactor culture runs. pH was monitored by an in line probe. At the end of the culture run, cells were harvested via trypsinization.

Gene and surface marker expression

A panel of antibodies against cell surface proteins was used for analysis with a Guava® easyCyte™ 8HT 2 laser, 8 channel flow cytometer. Gene expression was assessed with qRT-PCR using Custom PCR Array and PerfeCTa SYBR Green SuperMix. 1 µg total RNA was used for cDNA synthesis prior to the RT-PCR reaction. The ΔΔCT method was used to quantify the relative gene expression by normalizing to housekeeping genes (GAPDH and RPL13A).

Differentiation assays

hMSCs harvested from Mobius® CellReady bioreactors were differentiated into adipocytes and osteocytes using kits obtained from EMD Millipore. Undifferentiated controls were cultured for the duration of the experiment in growth medium lacking differentiation factors. Cells were fixed, stained (Oil Red O solution for adipocytes, Alizarin red S for osteocytes) and imaged according to the manufacturer's protocol.

System Advantages

Platelet Lysate

- Rich in cytokines and growth factors
- Sourced from expired, AABB pathogen -tested, human blood donations
- Multiple donors pooled for consistency
- Source material approved for human use
- Low-risk supply chain
- Xeno-free

3L Mobius® CellReady Bioreactor

- Rapid, robust expansion of MSCs
- Lower media volumes required in comparison to multilayer culture
- Controlled environment
- Single-use
- Scalable

Results

Figure 1: Equivalent cell yield with PLTMax Human Platelet Lysate versus FBS

hMSCs derived from two different adult bone marrow donations (hMSC1 or hMSC3) were expanded in 3L bioreactors in DMEM supplemented with either 5% PLTMax hPL or 10% FBS. Despite distinct growth patterns, PL supported similar cell yields in comparison to FBS for both cell lines.

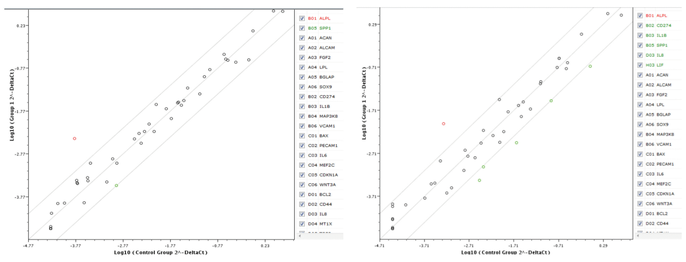


Figure 2: Equivalent gene expression with PLTMax Human Platelet Lysate versus FBS

RNA was isolated from hMSCs expanded in 3L bioreactors in DMEM supplemented with either 5% PLTMax hPL or 10% FBS, and analyzed for the expression of 46 MSC-relevant genes. Of the genes examined, 96% had high correlation between the two conditions in hMSC1 cells (left), whereas 87% were highly correlated in hMSC3 cells (right).

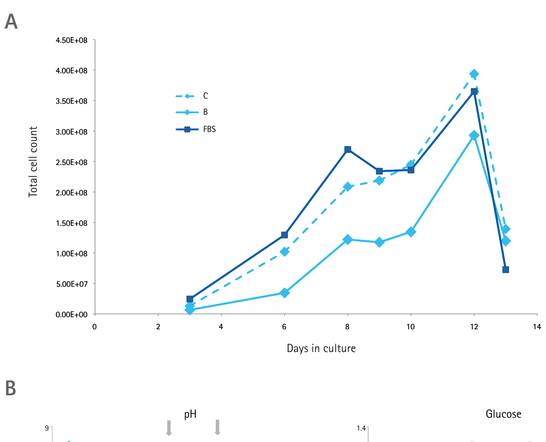


Figure 3: PL sample C supported higher hMSC growth than sample B

hMSCs (hMSC1) were expanded in 3L bioreactors with two PL preparations, samples B (+Heparin) and C (-Heparin). Better cell yields were obtained with DMEM supplemented with 10% sample C in comparison to 10% sample B (A). Nutrient / metabolite profiles of bioreactor cultures. Arrows indicate feeds days (B).

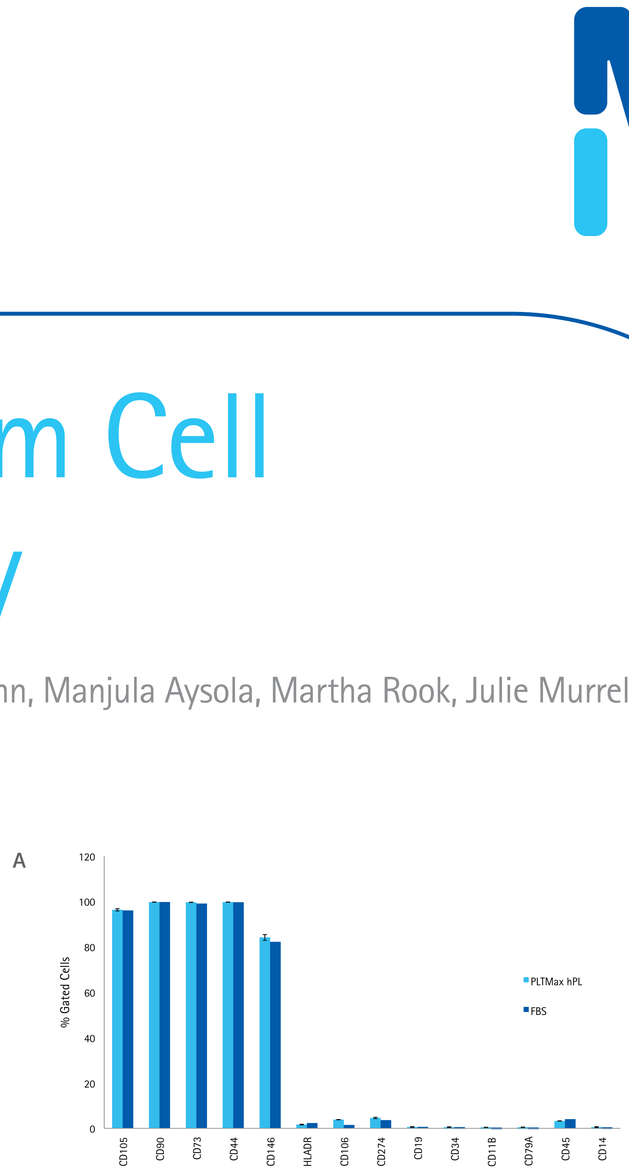


Figure 4: Biomarker Expression was maintained on hMSCs expanded with PL in comparison to FBS

Flow cytometry analysis for cell surface markers was performed following 14 days expansion of hMSCs (hMSC1) in bioreactors. Equivalent marker levels were detected on hMSCs expanded with 5% PLTMax hPL in comparison to 10% FBS (A), as well as 10% samples B and C in comparison to 10% FBS (B).

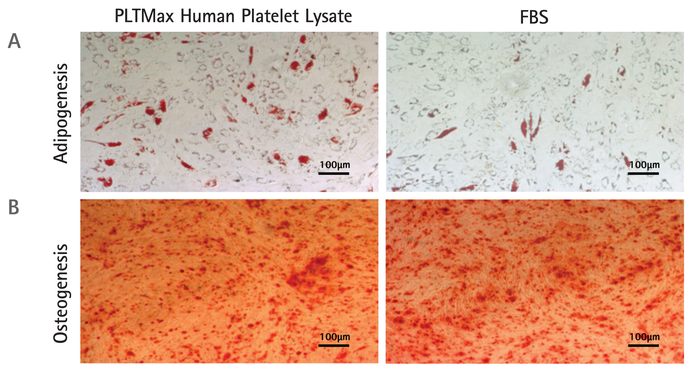


Figure 5: hMSCs retain the potential to differentiate after expansion in 3L bioreactors in PL-containing medium

hMSCs (hMSC1) expanded for 14 days in 3L bioreactors in DMEM supplemented with 5% PLTMax hPL or 10% FBS were positive for the adipogenesis stain Oil Red (A) and for the osteogenesis stain Alizarin Red (B) following the respective differentiation protocols. hMSC1 cells expanded in the bioreactor, but left undifferentiated, were negative for both stains (not shown).

Summary

- The global serum supply will not be able to support the growing needs of the cellular therapy industry.
- Platelet lysate (PL) is a candidate serum alternative for stem cell expansion under xeno-free conditions.
- Three PL samples from varied vendors and different heparin requirements were evaluated in comparison to FBS supplementation:

Sample	Growth in CellReady	Gene Expression	Surface Marker Expression	Differentiation Potential
PLTMax hPL	Equivalent	Equivalent	Equivalent	Equivalent
B	Slightly less	Not tested	Equivalent	Not tested
C	Equivalent	Not tested	Equivalent	Not tested

- PL supported hMSC expansion in the Mobius® 3L CellReady bioreactor, while retaining stem cell properties

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