Development of Standard Protocol for the cGMP Production of Human Mesenchymal Stem/Stromal Cells from Different Source Tissues.

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Introduction

Mesenchymal stem/stromal cells (MSC) constitute a heterogeneous subset of regenerative cells which can be harvested from several adult tissues. Initially MSC were identified in bone marrow but currently they can be harvested from several autologous and allogeneic sources, including adipose tissue, peripheral blood, lungs, marrow spaces of long bone, synovial fluids, periodontal ligament, dental pulp and muscle. In addition, MSCs are also obtained from placenta, umbilical cord and cord blood. Notably, MSCs obtained from various sources differ in their biological characteristics, CFU-F efficiency, specific markers, multi-lineage differentiation as well as paracrine functions which may determine their different clinical applications.

The use of MSC from different sources together with the fact that most laboratories use their own standardized protocols for cell expansion results in a clear variability in cell preparations between labs which irremediably leads to variability in the outcome of experiments and clinical trials across different laboratories and institutions. Investigations have attempted to address this issue of unpredictable outcomes by seeking to establish standard practices for the isolation, characterization, and maintenance of cells in culture. The development of uniform protocols for the expansion and preparation of MSCs is crucial to improve comparison of results, for the exploitation of MSC clinical potential and for a successful translation of approaches to the clinic.

Here we present the development of a standardized protocol for a consistent and efficient cGMP production of clinically relevant numbers of MSCs obtained from different source tissues, using PLTGold®, a fully xenogeneic free human platelet lysate.

Results

I. Human Platelet Lysates (hPL) PLTMax® and PLTGold® for growth of MSCs

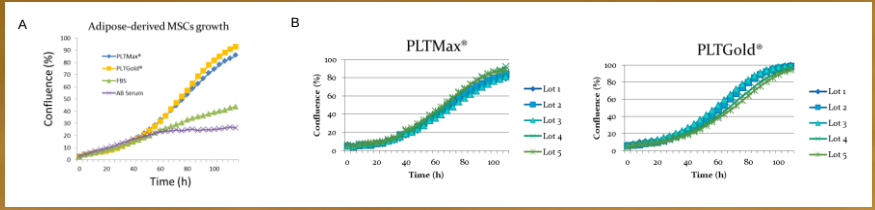


Figure 1. Use of hPL for growth of adipose-derived MSC. A) Comparison between hPL (PLTMax® and PLTGold®) and serum based products in a cell growth assay. B) Lot to lot consistency for 5 representative lots of PLTMax® and PLTGold®.

We have previously shown how using PLTMax® (animal serum free hPL) or PLTGold® (completely xenogeneic free hPL) as media supplements to grow adipose-derived MSCs (Ad-MSCs), we can achieve a significantly increased cell growth (reduced cell doubling times) in comparison with FBS or Human AB Serum (Figure 1A). Our production process allows for a high lot to lot consistency as reflected on the cell kinetics outcome for different lots of the product (Figure 1B).

II. Selection of proper basic medium and hPL for optimal growth of MSCs from different tissue sources

MSCs from different tissue sources have different growth characteristics and nutrient demand. To develop a standard protocol that allows for an efficient expansion of MSCs regardless of origin, it is necessary to use a rich basic medium. In figure 2A we can see how cell kinetics differ between MSCs from 3 different origins (adipose tissue, bone marrow and umbilical cord) when grown in two different basic media supplemented with PLTMax®. Doubling times for umbilical cord derived MSCs (UC-MSCs) are significantly higher (average 40h) than for Ad-MSCs or Bone Marrow derived MSCs (BM-MSCs) (average 25h) when grown in the same mid-level basic media supplemented with 5% PLTMax®. However, the use of PLTGold® instead of PLTMax® combined with richer media results in a significant decrease in doubling times for UC-MSCs, obtaining around 80% confluence in 6 days of culture (Figure 2B) and doubling times of around 30h. BM-MSCs also showed decreased doubling times when using PLTGold® *vs* PLTMax® (Data not shown) and seemed to be the most sensitive to type of basic media amongst the studied MSCs (Figure 2A). For Ad-MSCs, the difference between cell growth using PLTGold® *vs* PLTMax® is not significant (Figure 1A). The difference between using different basic media did not become apparent until a large scale expansion of Ad-MSCs was conducted, as observed in Figure 2C.

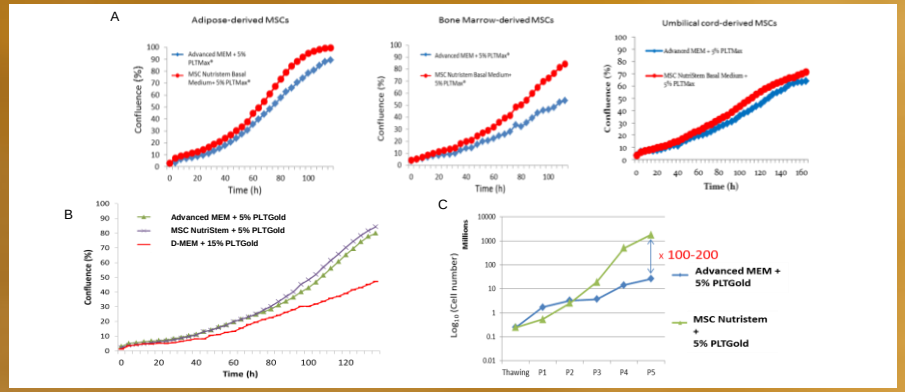
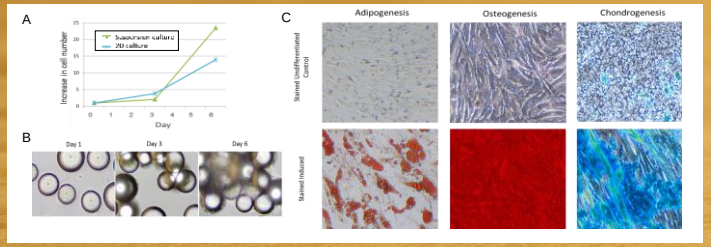


Figure 2. Growth of MSCs from different tissue sources. A) Comparison between Ad –MSCs, BM-MSCs and UC-MSCs grown with MSC Nutristem Basic medium or Advanced MEM, both supplemented with PLTMax®. B) Growth of UC-MSCs using MSC Nutristem Basic medium, Advanced MEM or D-MEM supplemented with PLTGold®. C) Cell yield for Ad-MSCs grown for 2 weeks (5 passages) on 2D monolayers using MSC Nutristem Basic medium or Advanced MEM, both supplemented with PLTGold®.

III. Large scale expansion of MSCs in bioreactors

After trying different microcarriers from different manufacturers, we found that the Star Plus microcarriers from Pall Biotech offered the best support for the expansion of Ad-MSC in suspension using small vertical wheel bioreactors (PBS Biotech) (Figure 3B) . More than 8x107 cells were obtained in just 6 days of culture in suspension (over 23 times the initial cell feed) when using MSC Nutristem supplemented with PLTGold®. We observed a significant increase on Ad-MSC cell number respect to initial seeding density for cells grown in suspension using microcarriers *vs* in 2D systems (T175 flasks) (Figure 3A). Ad-MSC expanded in suspension using medium supplemented with PLTGold® continued to demonstrate capacity to undergo adipogenesis, osteogenesis and chondrogenesis .

Figure 3: Ad-MSCs large scale growth in suspension. A) Increase on Ad-MSCcell number respect to initial seeding density for cells grown in suspension using microcarriers *vs* in 2D systems (T175 flasks). B) Imaging of Ad-MSCs growing on Star Plus microcarriers (Pall Biotech) at different times after seeding. C) Differentiation of Ad-MSC expanded in suspension using medium supplemented with PLTGold Adipogenesis (Oil-red-O staining), osteogenesis (Alizarin Red staining) and chondrogenesis (Alcian blue staining) shown.



Conclusions

-PLTMax® and PLTGold® hPLs exceed the performance and consistency of serum-based supplements, with higher yield and healthier cells

-The use of a richer medium allows for the use of lower percentages of supplementation and the production of higher number of cells of interest in less amount of time: more cost effective.

-We can large amounts of Ad-MSCs in suspension when using the appropriate combination of basic medium, hPL and microcarriers.