Rapid expansion of Mesenchymal Stem/Stromal Cells using optimized media supplemented with human platelet lysate PLTMax® or PLTGold®, suitable for cGMP expansion at large scale

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Introduction

The effective transfer into the clinic of allogeneic cell therapies using MSCs will depend predominantly on the development of large scale and cost effective manufacturing platforms that allow production of functional cells at the scale required to meet clinical demand. Here we present the results of a study to develop a protocol for the establishment of large scale expansion of MSCs in bioreactors using our first and second generation human platelet lysates (hPL) PLTMax® and PLTGold® and evaluating different basic media and a panel of 9 microcarriers.

Results and discussion

I. hMSCs growth kinetics using platelet lysate

Real time imaging of adipose-derived MSCs growth using PLTMax® or PLTGold® as a media supplement showed increased cell growth kinetics (reduced cell doubling times) compared to cells grown in medium supplemented with FBS or Human AB Serum (Figure 1 A and B). Similar results were obtained for bone marrow-derived MSCs (data not shown).

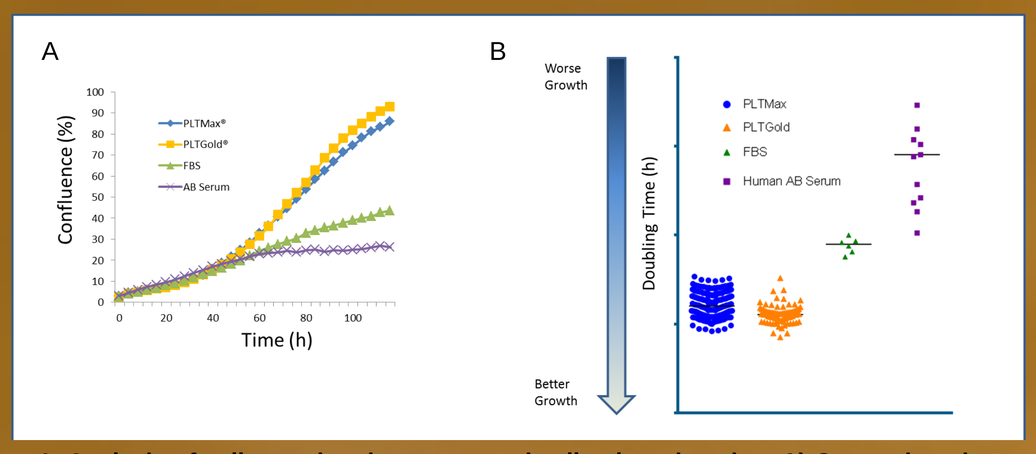

Figure 1: Analysis of cell growth using automated cell culture imaging. A) Comparison between A) cell kinetics and B) doubling times of adipose-derived MSC cultured in medium supplemented with either PLTMax®, PLTGold®, FBS or Human AB Serum.

When conducting a large scale expansion of MSCs it is very important to find the right combination between media supplement and basal media. In a 5 day culture comparing Advanced MEM (Gibco) and MSC NutriStem® XF Basal medium (Biological Industries), both supplemented with PLTMax®, using adipose-derived and bone marrow-derived MSCs, we observed a significant increase in cell growth when using NutriStem® XF Basal medium *vs* Advanced MEM (Figure 2 A). At day 4 of culture, we had over 2 times more cells in the plates with MSC NutriStem® XF Basal medium supplemented with PLTMax® with respect to the plates with Advanced MEM supplemented with PLTMax® (Figure 2 B) or PLTGold® (data not shown).

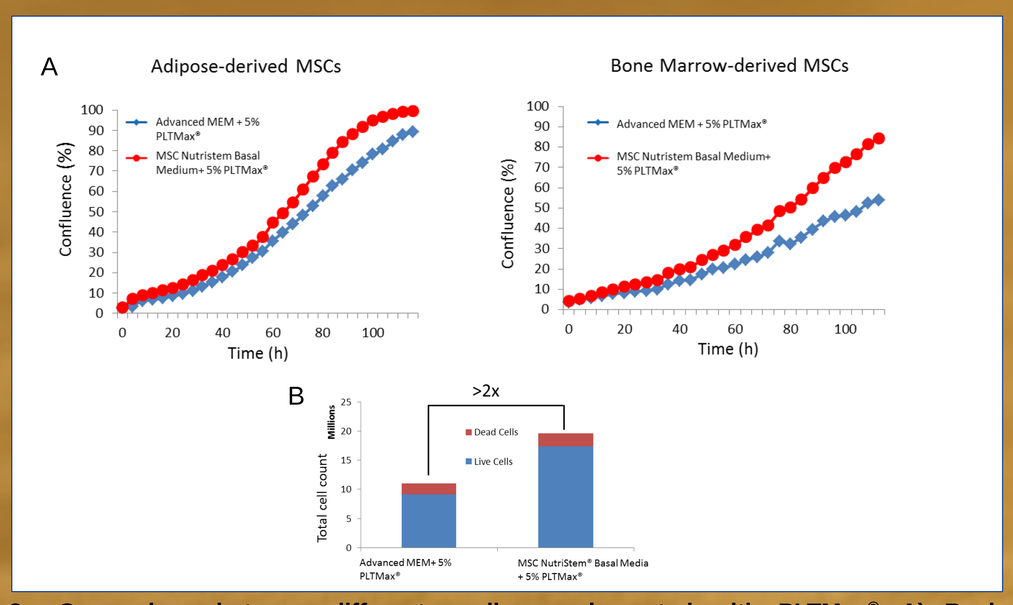

Figure 2: Comparison between different media supplemented with PLTMax®. A) Real time monitoring of adipose-derived and bone marrow-derived MSCs cultured in different medium supplemented with PLTMax®. B) Total cell number obtained for adipose-derived MSCs at day 4 of culture with different medium supplemented with PLTMax®.

II. Large scale expansion of hMSCs in 2D culture

Using media supplemented with PLTMax® or PLTGold®, cells were passed every 2-3 days for a total of 2 weeks. We found that MSC NutriStem® XF Basal Medium supplemented with PLTMax® or PLTGold® exceeds the performance of Advanced MEM supplemented with the same hPLs, obtaining up to $2x10^{10}$ cells (200 times more cells) in only 5 passages (Figure 3A). After performing a long term expansion for a total of 12 passages in MSC NutriStem® XF Basal Medium supplemented with PLTMax® (data not shown) or PLTGold® (Figure 4), adipose-derived and bone marrow-derived MSCs still maintained multipotency, with capacity to undergo adipogenesis, osteogenesis and chondrogenesis (Figure 4 A and B), as well as MSC phenotype (Figure 4 C).

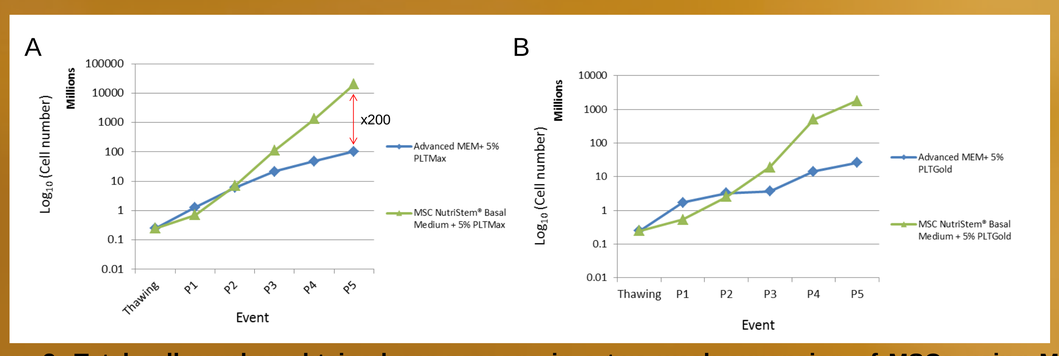

Figure 3: Total cell number obtained per passage in a two week expansion of MSCs using MSC NutriStem® XF Basal Medium and Advanced MEM medium supplemented with A) PLTMax® or B) PLTGold®.

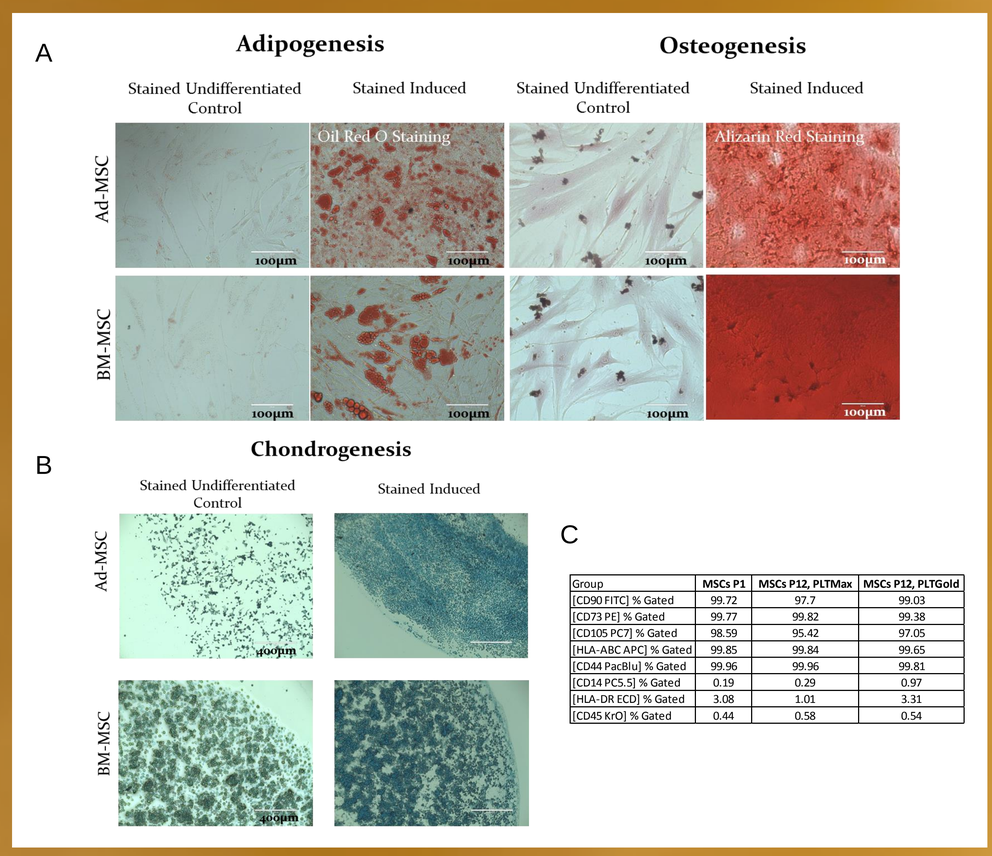

Figure 4: A) Adipogeneis (Oil-red-O staining), osteogenesis (Alizarin Red staining) and chondrogenesis (Alcian blue) from adipose derived MSCs (Ad-MSC) and bone marrow-derived MSCs (BM-MSC) passaged 12 times in medium supplemented with PLTGold®.

III. Large scale expansion of hMSCs in bioreactors

Different microcarriers were evaluated in small-scale six-well plate screening studies to determine biocompatibility with adipose-derived MSCs: Cytodex™ 1 and Cytodex™ 3 (GE), Vitronectin XF™ (Primorigen Biosciences), Plastic, Plastic Plus, Star Plus, Collagen Coated, Fact III and Hillex II (Pall SoloHill®). Hillex II did not show a satisfactory cell binding. The rest of the microcarriers, which showed satisfactory cell binding, were tested in suspension culture in spinner flasks stirred continuously at 35 rpm, using MSC NutriStem® XF Basal Medium supplemented with PLTMax® or PLTGold® and a concentration of microcarriers according to each manufacturer's specifications. 50% of the medium was replaced every 3 days. The best growth rates were found with the Collagen Coated and Star Plus (Both of them with a plastic core) from Pall SoloHill® as well as with the Vitronectin XF™ (Primorigen Biosciences). Cells cultured with the collagen coated microcarriers (Figure 5 B) and Vitronectin XF™ (Figure 5 C and D) showed similar growth rates to cells cultured with the same media in 2D systems, whereas cells cultured with the Star Plus microcarriers showed higher growth rates than monolayer cultures, obtaining up to $4.5x10^7$ cells in just 6 days (over 23 times the initial cell feed) (Figure 5 A and B).

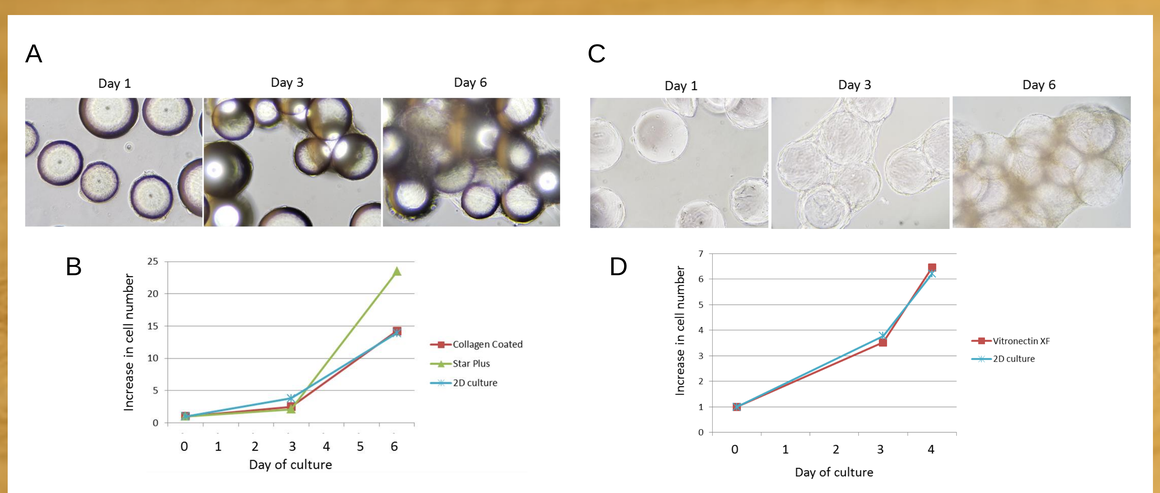

Figure 5: Cell attachment and growth on microcarriers. 200x pictures of cells attached and growing on A) Star Plus microcarriers and on C) Vitronectin XF™ microcarriers at day 1, 3 and 6 after starting the culture on spinner flasks with MSC NutriStem® XF Basal Medium supplemented with PLTGold®. B) Increase in cell number respect to the cell feed for cells grown on B) Collagen coated and Star Plus microcarriers from Pall SoloHill® and on D) Vitronectin XF™ microcarriers from Pall SoloHill® in comparison with cells grown in culture flasks.

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