

Introduction

Mesenchymal stem cells, also referred to as multipotent mesenchymal stromal cells or MSCs, hold extraordinary potential for use in therapeutic applications, notably due to the wide accessibility of source material from various tissue types, the cells' ready expansion in culture, and their capacity for multipotent differentiation in vivo. Additionally, MSCs have shown to possess unique biological properties that stimulate the recovery of inflamed or injured tissue, which help to enable regeneration of tissues and organs and/or suppression of immune-related diseases. According to clinicaltrials.gov, there are over 500 current or concluded clinical trials using MSCs today worldwide.

Since MSCs must first be expanded ex vivo in order to reach appropriate numbers for cell-based therapies, the quality of cell culture medium and its performance is particularly crucial in therapeutic applications. Historically, the majority of MSC culture media contain animal-derived serum, most often fetal bovine serum (FBS). The use of animal-derived serum in cell therapy applications is not ideal due to the highly inconsistent and undefined nature of the serum, the exposure of the cells to xenogenic proteins, and obvious safety concerns for patients. The impact on safety, reproducibility, and overall cost are some of the convincing reasons why many labs are seeking alternatives to traditional FBS-based solutions.

With the increasing demand for high-quality reagents for cell therapy and regenerative medicine, PLTMax® Human Platelet Lysate is an ideal alternative to FBS and other serum supplements. PLTMax® is animal serum-free, highly effective in MSC culture, and has a proven track record in clinical use. PLTMax® is generated from platelets that were intended for use in hospitals, and is currently used in over 30 Phase I through Phase III clinical trials in the US, Europe, and Asia. When PLTMax® is used as a culture supplement in the highly optimized, defined, xeno-free MSC NutriStem® Basal Medium, the combination creates an excellent complete medium for the routine MSC culture as well as large-scale expansion of MSCs for translational medicine.

Materials

MSC NutriStem® Basal Medium

Biological Industries USA, Catalog Number: 05-201-1A

PLTMax® Human Platelet Lysate

Biological Industries USA, Catalog Number: PLTMax100

Conclusion

The elimination of animal serum and transition to more optimal media formulations holds many advantages for cell culture, especially in the field of regenerative medicine and cellular therapy. The use of MSC NutriStem® Basal Medium supplemented with 5% PLTMax® Human Platelet Lysate is an ideal solution for large-scale expansion of human MSCs. This unique, clinically relevant, animal serum-free medium combination expands MSC successfully, with a healthy phenotype and excellent growth kinetics, far exceeding the performance of the same medium supplemented with FBS or human AB serum.

Summary

- **PLTMax® Human Platelet Lysate is an animal serum-free media supplement that has been used in over 30 Phase I to Phase III clinical trials worldwide.**
- **PLTMax® as a culture media supplement exceeds the performance of serum-based supplements to culture human MSCs.**
- **MSC NutriStem® Basal Medium supplemented with PLTMax® offers an excellent combination for large-scale expansion of MSCs for both therapeutic and research use.**
- **Both the MSC NutriStem® Basal Medium and PLTMax® are produced under cGMP guidelines and have accepted Drug Master Files with the FDA for accelerated use in cell therapy applications.**

Experimental Results

PLTMax® is consistent lot-to-lot. PLTMax® Human Platelet Lysate is generated from a large pool of platelets from multiple donors, which are sourced and manufactured to produce a consistent and reliable culture supplement with very low lot-to-lot variability. The consistency in the reagent is reflected not only in the observed cell kinetics (**Figure 1A**), but also in many other parameters analyzed, including consistent levels of FGF, VEGF, PDGF-AB, and PDGF-BB, measured across numerous batches of PLTMax® (**Figure 1B**).

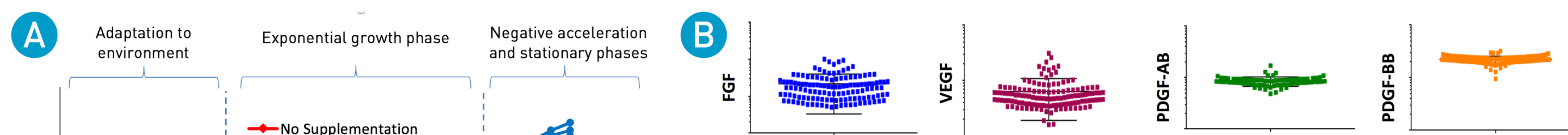


Figure 1: PLTMax® demonstrates high lot-to-lot consistency. A) Minimal lot-to-lot variation seen by real-time imaging of cell growth of adipose-derived MSCs across 5 lots of PLTMax® medium supplement. The growth of each PLTMax® lot is plotted according to the time in culture and the increase in cell density as measured by cell confluence. The difference between performance of cells cultured in PLTMax® compared to human AB serum is observed as early as 36 hours in culture. B) Consistency of growth factor levels measured in over 100 lots of PLTMax®.

PLTMax® out-performs serum supplements. Cell kinetics of both adipose-derived and bone marrow-derived MSCs were analyzed when MSCs were cultured in MSC NutriStem® Basal Medium supplemented with either PLTMax®, human AB serum, or FBS. In both cases, MSC NutriStem® Basal Medium supplemented with 5% PLTMax® exceeded the performance of the same basal medium supplemented with either of the serum-based supplements, with notable differences in cell confluency seen by day 3 (**Figure 2**). Additionally, MSCs cultured in MSC NutriStem® Basal Medium and 5% PLTMax® maintained a healthier morphology than those cells cultured with media containing serum-based supplements (**Figure 3**).

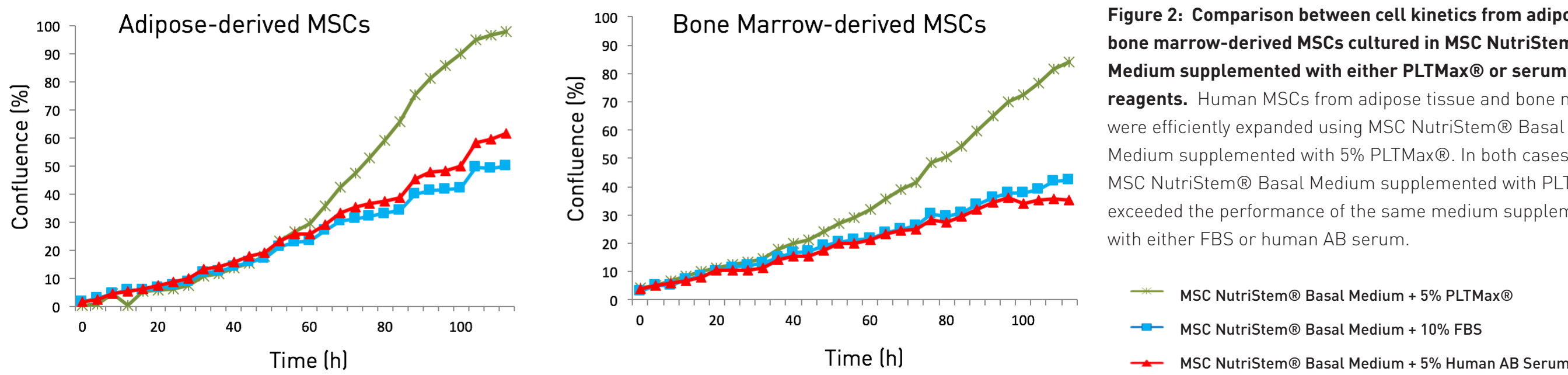
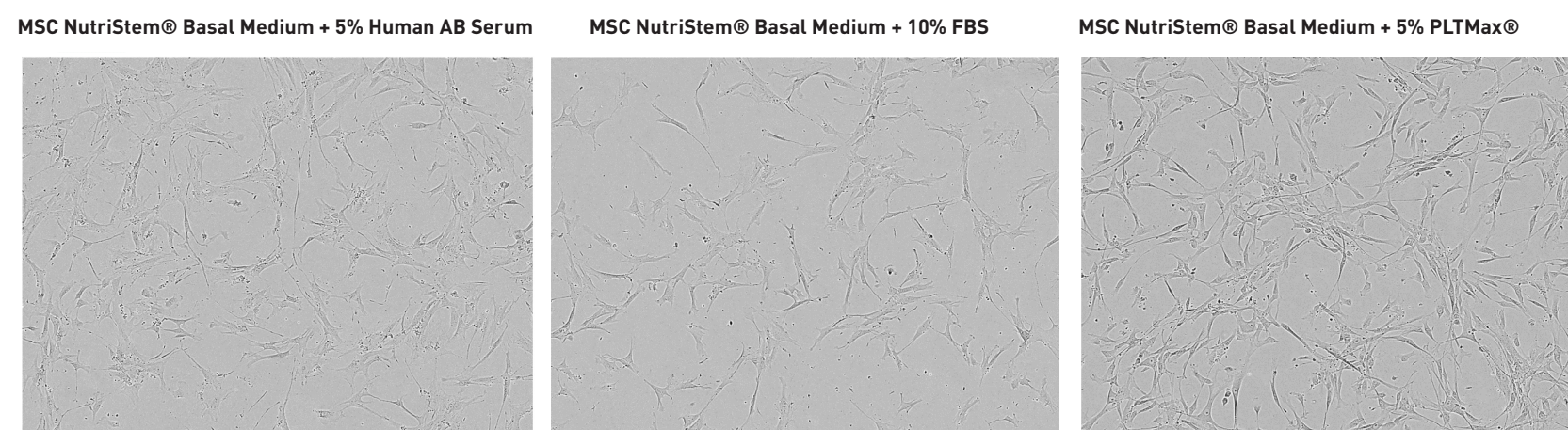


Figure 2: Comparison between cell kinetics from adipose- and bone marrow-derived MSCs cultured in MSC NutriStem® Basal Medium supplemented with either PLTMax® or serum-based reagents. Human MSCs from adipose tissue and bone marrow were efficiently expanded using MSC NutriStem® Basal Medium supplemented with 5% PLTMax®. In both cases, MSC NutriStem® Basal Medium supplemented with PLTMax® exceeded the performance of the same medium supplemented with either FBS or human AB serum.

Figure 3: MSC NutriStem® Basal Medium supplemented with PLTMax® maintains normal, healthy MSC morphology.

Images of adipose derived-MSCs at day 3 post-thaw show that cells grown in medium supplemented with PLTMax® have a healthy phenotype, with characteristic small, long, thin cell body with a few cell processes.



MSC NutriStem® Basal Medium improves cell recovery from thaw and MSC expansion in culture.

In addition to the observed improvement in cell morphology and kinetics, MSCs thawed and cultured with media supplemented with PLTMax® showed better attachment and viability after cryopreservation. The basal medium used to culture the cells had a measurable impact on the cells' recovery. Cells cultured with MSC NutriStem® Basal Medium supplemented with 5% PLTMax® exhibited a cell yield over 10 times higher than cells cultured with the same supplement used in Advanced MEM medium, which is historically one of the most widely used basal media for MSC culture (**Figure 4**).

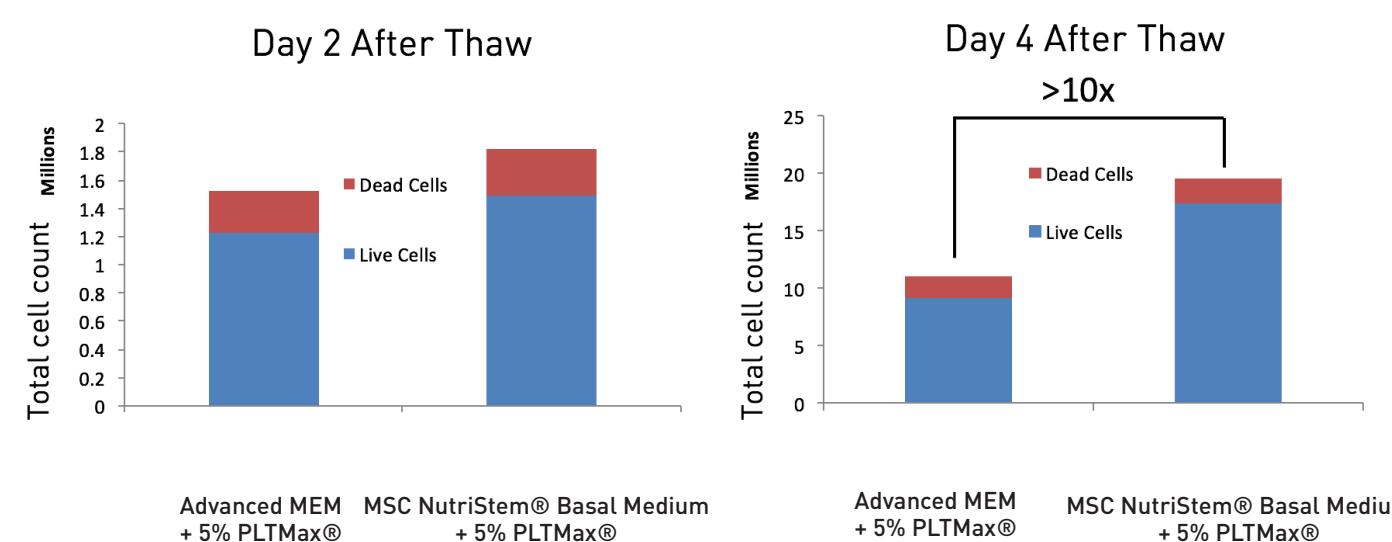


Figure 4: MSC NutriStem® Basal Medium improves cell viability and total cell count post-thaw.

Cell viability and total cell number of adipose-derived MSCs was measured at 2 and 4 days after thawing. After only 4 days, more than 10 times more cells were obtained when the MSCs were cultured in MSC NutriStem® Basal Medium supplemented with PLTMax® as compared to Advanced MEM supplemented with PLTMax®.

Higher MSC yields are obtained using MSC NutriStem® Basal Medium supplemented with PLTMax®. After the initial recovery from thaw, the exceptional growth and proliferation of MSCs cultured in MSC NutriStem® Basal Medium supplemented with PLTMax® continues to outperform cultures maintained in traditional Advanced MEM supplemented with PLTMax®. Improved cell proliferation is observed within the first passage. However, subsequent passages show proliferation rates in MSC NutriStem® Basal Medium and PLTMax® that soon exceed Advanced MEM and PLTMax® by a difference of 10 billion cells (**Figure 6**), making this complete medium ideal for large-scale expansion of MSCs.

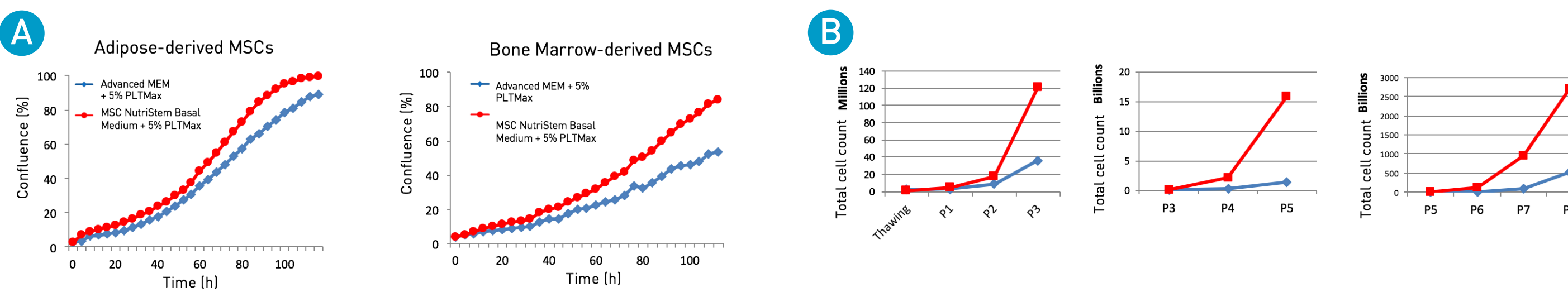
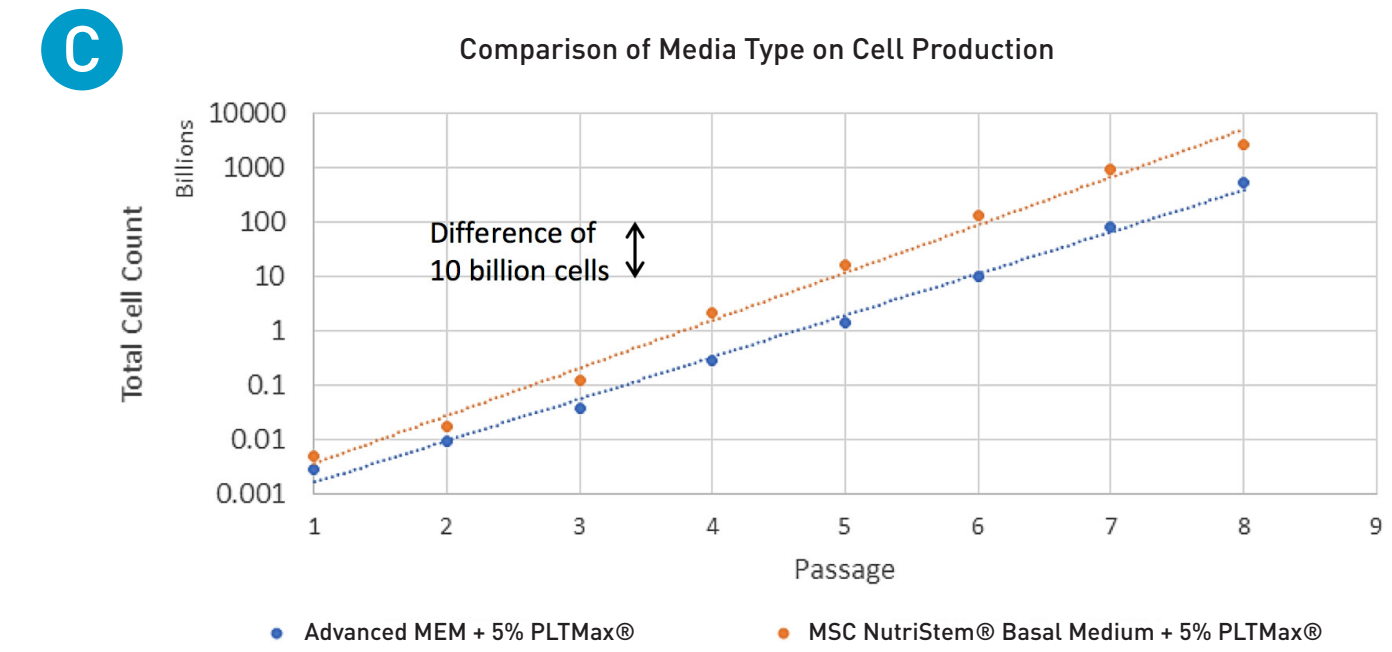


Figure 5: Higher cell yields are obtained using MSC NutriStem® Basal Medium supplemented with PLTMax®.

A) Improved cell growth kinetics measured by % confluency of adipose- and bone marrow-derived MSCs in MSC NutriStem® Basal Medium supplemented with PLTMax® or Advanced MEM supplemented with PLTMax® is seen within the first 5 days in culture. B) With subsequent passages, the difference between cell growth and proliferation become more pronounced, as shown by the expansion rate of adipose-derived MSCs. C) Over the course of 8 passages post-thaw, adipose-derived MSCs cultured in MSC NutriStem® Basal Medium supplemented with 5% PLTMax® continue to maintain containing over 10 billion more cells than the same cells cultured in Advanced MEM supplemented with 5% PLTMax®.



MSC NutriStem® Basal Medium supplemented with 5% PLTMax® is an exceptional medium for MSC expansion for therapeutic use.

Both the basal medium and supplement are critical components of an optimized MSC medium, and finding the right combination can have a significant impact on the cells in culture. The combination of MSC NutriStem® Basal Medium and PLTMax® creates an excellent complete medium for routine culture and large-scale expansion of healthy, proliferative MSCs in vitro (**Figure 6**).

Figure 6: MSC NutriStem® Basal Medium supplemented with PLTMax® is an exceptional medium for MSC expansion for therapeutic use.

Adipose-derived MSCs cultured in MSC NutriStem® Basal Medium supplemented with 5% PLTMax® maintain a healthy phenotype while exhibiting rapid cell growth and proliferation over the course of a typical passage cycle.

