# Comparative study on the expansion and characterization of bone marrow derived hMSCs for clinical relevancy

## Summary

Mesenchymal stromal cells (MSCs) have emerged as a new player in the field of regenerative medicine due to their potential therapeutic applications. Unfortunately, extensive in vitro culturing and expansion is required to obtain adequate numbers of bone marrow-derived MSCs (BM-MSCs). MSCs are traditionally cultured in various basal media supplemented with fetal bovine serum (FBS). However, there are safety concerns regarding the use of FBS for clinical applications. FBS is an ill-defined supplement which results in lot to lot variation. The use of FBS is discouraged by regulatory agencies due to the risk of contamination with microbiological contaminants and xenogenic compounds which may influence cell behavior. Furthermore, the collection of FBS may be considered ethically inhumane.

A new study published in Scientific Reports takes a comparative look at six commercially available low serum or serum-free media in the growth and expansion of bone marrow-derived MSCs and compared the MSC culture characteristics and functions with that of a serum-containing medium (FBS). Authors conducted a series of experiments to assess the ideal conditions for the characterization and expansion of BM-MSCs for cell-based research applications.

## The Spotlight

**Research area:** General culture, expansion and characterization of human, BM-MSCs

**Cell type(s):** BM-MSCs

**Experiment purpose:** A comparative look at six commercially available media to determine the ideal conditions for low-serum or serum-free cell manufacturing of BM-MSCs.

## Product Highlights

**PLTMax® Human Platelet Lysate**

- Contains more than 100 growth factors and proteins
- Enhances genetic stability in stem cell cultures
- Used in more than 30 clinical trials worldwide
- FDA Drug Master File available

## Experiment overview

**Isolation of BM-MSCs**

- Bone marrow was aspirated from healthy donors between 18 and 40 years of age.
- BM-MSCs were cultured in a serum-containing medium up to passage three.
- Cell banks were produced by pooling three different donors, thereby reducing individual variability.

**Sub-culturing and expansion**

- Cryopreserved BM-MSCs were thawed, centrifuged, and resuspended in the control medium.
- Cells were seeded at low and high seeding densities (1000 and 5000 cells/cm$^2$) in each culture medium by employing the passive adaptation method.
- Media change was performed at 55–60% confluency and cells were harvested at 80–90% confluence.

**Summary of comparative experiments conducted**

- Population doubling and doubling time
- Immunophenotype analysis
- Tri-lineage differentiation capacity
- Quantitative gene expression analysis
- Colony forming unit-fibroblast
- VEGF secretion and lymphoproliferation assay
- Indoleamine 2,3 dioxygenase activity assay

## Results

**Expansion analysis and comparative look at cell size, morphology, and proliferation kinetics**.
Although the media tested supported the growth of BM-MSCs at a low seeding density and little differences were observed in the expression of MSC-specific markers, a clear difference among the media was observed in population doubling time, cell yield, potency, colony-forming ability, differentiation potential, and immunosuppressive properties.

Researchers showed that BM-MSCs cultured with PLTMax® Human Platelet Lysate displayed an average cell diameter similar to the serum containing medium (control), while the five other media tested produced on average larger cells that were similar in size to one another with an average cell diameter higher than the control medium. BM-MSCs cultured in PLTMax® Human Platelet Lysate were spindle-shaped, elongated, bright with tapering ends comparable to control cultures and did not result in cell aggregation contrary to other media tested (Figure 1).

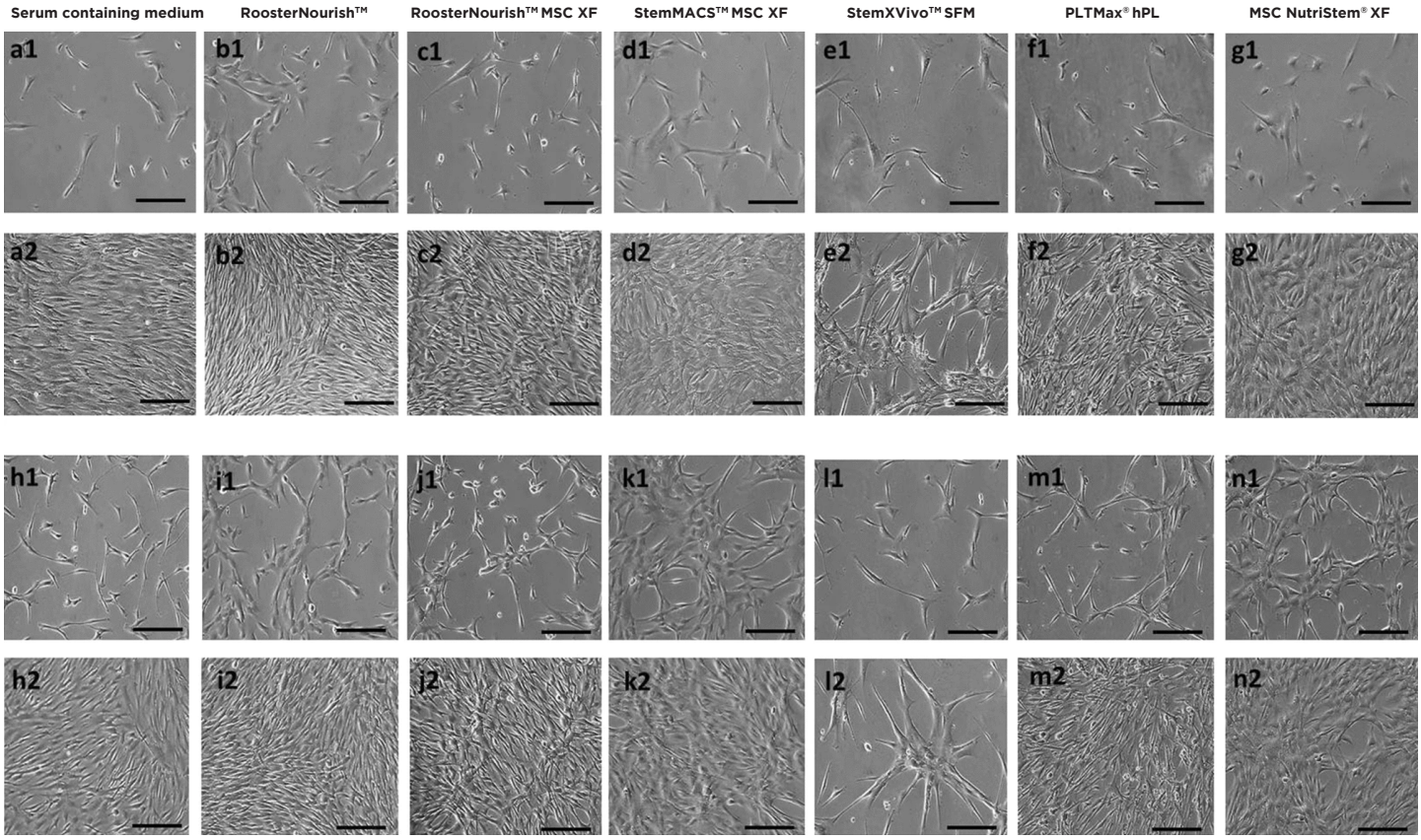


**Figure 1. Morphology images of BM-MSCs.** BM-MSCs were cultured in various low-serum and serum-free culture conditons at a seeding density of 1000 cells/cm$^2$ and 5000 cells/cm$^2$. Cells were expanded to passage 5. Most microphotographs shown at 10–25% confluency (1) and 80–90% confluency (2). Experiments were performed in duplicate and the representative images are shown. Please see the publication for a complete review of the data shown.

**Crucial VEGF secretion of BM-MSCs**

Secretion of growth factors or cytokines is one of the characteristics of MSCs that plays a crucial role in cell engraftment, neovascularization, and wound healing which may be directly linked to the potency of MSCs. Studies have shown that high levels of VEGF improve the therapeutic efficacy of MSCs. MSCs cultured in PLTMax® secreted significantly higher amounts of VEGF when compared to other media and control medium (Figure 2).

**First comprehensive low and serum-free media study**

In summary, this is the first comprehensive study comparing the culture conditions of BM-MSCs. Researchers showed that except for one media, all other media supported the growth of BM-MSCs at a low seeding density. No significant differences were observed in the expression of MSC specific markers among the various media tested. In contrary, the population doubling time, cell yield, potency, colony-forming ability, differentiation potential, and immunosuppressive properties of MSCs varied with one another. Findings also showed there were a few media conditions that required an extra step of surface coating that may not be feasible for large scale expansion in clinical applications.

**Two media conditions rise to the top**

Researchers concluded that two media conditions, including PLTMax® Human Platelet Lysate, were preferable in terms of cell yield, preserving MSC characteristics and reducing overall costs.

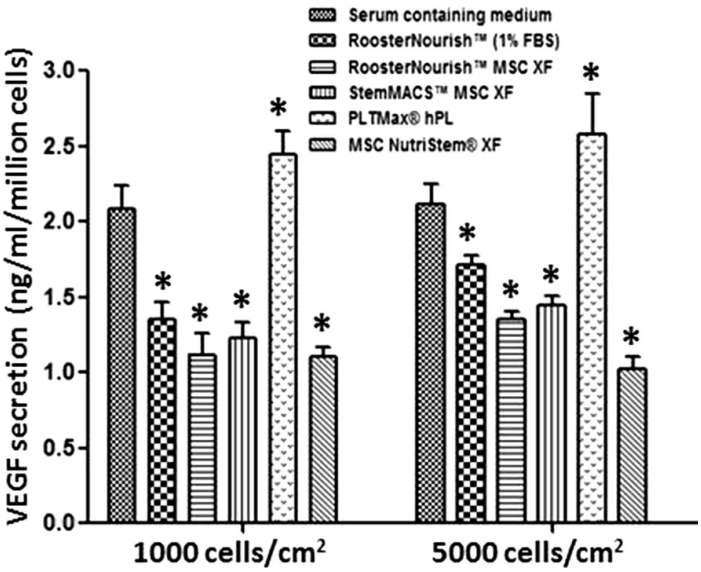


**Figure 2. Level of VEGF secretion of BM-MSCs**. Cryopreserved cells were seeded into T-75 flasks post revival and grown in respective media for 72 h and the amount of VEGF secreted was determined by ELISA. Results are expressed in concentration (ng/ml/million cells) a mean of duplicate experiments. The amount of VEGF secretion was higher in BM-MSCs cultured in PLTMax when compared to other media and the control medium.

## References

1. Bhat, S., et al. 2021. Expansion and characterization of bone marrow derived human mesenchymal stromal cells in serum-free conditions. Sci Rep 11, 3403. https://doi.org/10.1038/s41598-021-83088-1

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