

# Development of High Quality MSC and NSC For Human Disease Research

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## ABSTRACT

Quality and functionality of adult stem cells used in human disease research vary considerably due to non-standardized cell derivation protocols. Considerable variance exists with donor source, tissue processing, primary cultivation, and potency, and can generate results that are difficult to replicate and lead to inconclusive findings. To address this issue, we tested various approaches to optimize production of consistent lots of highly purified, rapidly expanding primitive adult stem cells under cGMP conditions that can be utilized as a cell standard or in disease research.

As hypoxia appeared to be beneficial for hMSC cultures (Ma et al 2009; Das et al 2010; Basciano et al 2011), we compared hMSC expanded under normoxic (20% O<sub>2</sub>) and hypoxic (5% O<sub>2</sub>) conditions. At Passage 2 and 4, hMSC yielded 2 fold and 1.5 fold increase in cell number when cultured under hypoxic condition as compared to normoxic condition. We also observed 1.7 fold increase in the number of colonies in CFU-F assays when cultured under hypoxic condition. Further expansion was realized when we incorporated media components with good lot-to-lot consistency and high unit activity.

Hypoxic hMSC should meet minimal criteria for multipotent MSC (Dominici et al 2006). Therefore, we analyzed hypoxic hMSC by flow cytometry for evidence of >95% MSC surface markers (CD73, CD90, CD105, CD166), <5% non-MSC surface markers (CD14, CD19, CD34, CD45), and <5% MHC class II marker (HLA-DR). At all passages, the hMSC exceeded minimal criteria. Differentiation studies confirmed ability of hypoxic hMSC to generate adipogenic, osteogenic and chondrogenic lineage cell types. In addition, a karyotype analysis was performed and no gross abnormalities were observed.

Since there is considerable interest in reparative capacity of hMSC, we performed a cytokine profile on normoxic and hypoxic hMSC. For angiogenic factor VEGF and chemoattractant factor SDF-1, we observed a significant increase under hypoxic condition versus normoxic condition (VEGF: 9.17±0.67 vs 7.67±0.86 ng/milCells/24 hours; SDF-1: 12.2±1.01 vs 9.2±1.27 ng/milCells/24 hours). These increases suggest that hypoxia endows hMSC with angiogenic- and migratory-inducing properties.

By employing an optimized cultivation and potency testing protocol during scale up expansion, we can start with 2 million cells from a Passage 2 master bank and yield 1.5 billion cells at Passage 4. This translates into a working bank size of 100 vials of 1.5e7 viable cells/vial within 14 days. We have established consistency of this system over forty lots of Passage 4 cells.

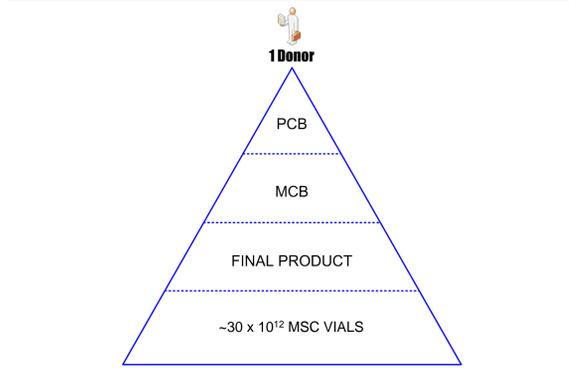
We have demonstrated the expansion of adult stem cells at larger scale following optimized standardized protocols without losing their intrinsic stem cell nature. With this source of well-characterized, consistent and potent adult stem cells, investigators can make meaningful comparisons in their disease research studies, drug screening assays, and design of effective cell-based therapies. Similar scale up approaches are being applied to human neural stem cells and human retinal pigment cells for the research.

These cells are for research use only.

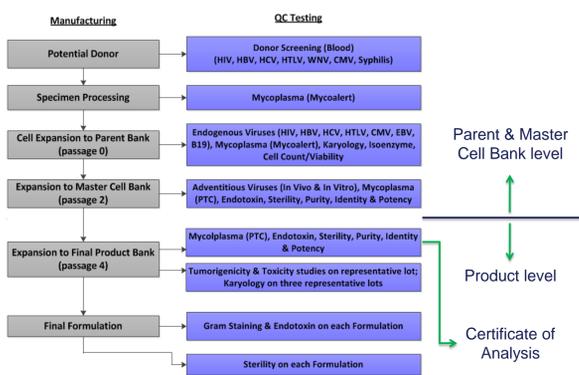


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## ADULT STEM CELL MANUFACTURING

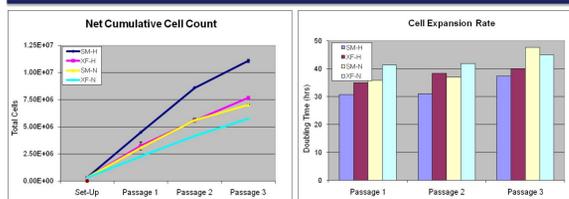


**Figure 1. Commercial Cell Factory Scale-Up**  
 Starting from a single bone marrow (BM) aspirate donor with informed consent, the Parent Cell Bank (PCB) of BM Mesenchymal Stem Cells (MSC) can be seeded in vessels with large surface areas (i.e. Cell Factories) and generate a Master Cell Bank (MCB) that is sufficient to manufacture final product of cryopreserved 30 x 10<sup>12</sup> vials with 1 x 10<sup>6</sup> cells/vial. Over 45 lots have been produced to date.

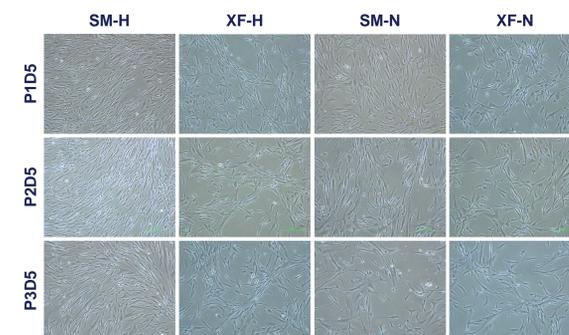


**Figure 2. Adult Stem Cell Manufacturing & Quality Testing Overview**  
 PCB are tested for adventitious agents, mycoplasma, karyotype and isoenzyme activity. In contrast, MCB is tested for adventitious agents, mycoplasma, endotoxin, sterility, purity, identity and potency (tri-lineage differentiation). In the final product (StemPro<sup>®</sup> BM MSC), all MCB assays except adventitious agents are performed; in addition, a sterility check is performed. Quality testing results may be obtained from product COA.

## MSC GROWTH & MORPHOLOGY

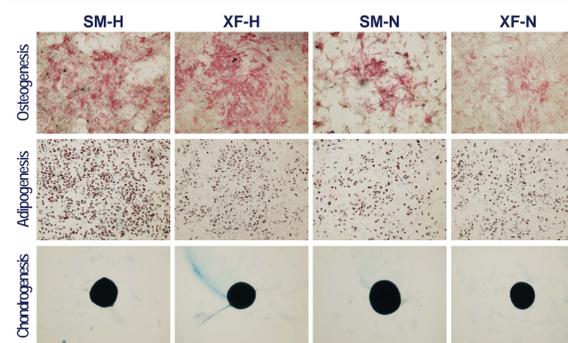


**Figure 3. Serum-Based & Xenofree Cell Culture Growth Kinetics**  
 Thawed and seeded at 4 x 10<sup>3</sup> cells/cm<sup>2</sup>, ≥90% viable StemPro<sup>®</sup> BM MSC was cultured in serum-containing media (SM) and StemPro<sup>®</sup> MSC SFM Xenofree media (XF) surface areas under hypoxic (5% O<sub>2</sub>) or normoxic (20% O<sub>2</sub>) culture conditions. BM MSC expands more rapidly under hypoxic condition than normoxic condition. SM-cultured cells exhibited slowed growth at third passage under normoxic condition. XF-cultured cells exhibit higher growth rate at higher seeding density (results not shown).



**Figure 4. Morphological Observations**  
 StemPro<sup>®</sup> BM MSC retains its fibroblast, spindle-like morphology in both SM and XF media at all passages.

## MSC POTENCY & IDENTITY

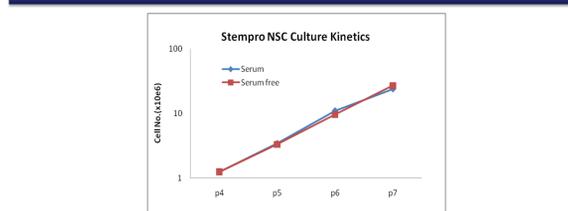


**Figure 5. Tri-Lineage Potential**  
 After recovery and subsequent 3 passages, StemPro<sup>®</sup> BM MSC maintained its tri-lineage potential after cultivation in SM and XF media under hypoxic and normoxic conditions. StemPro<sup>®</sup> Differentiation Kits were used to demonstrate MSC potency.

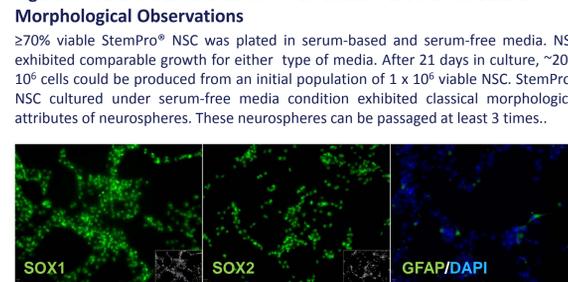


**Figure 6. Biomarker Analysis**  
 Flow cytometry analysis revealed that StemPro<sup>®</sup> BM MSC cultured in xenofree media under hypoxic/normoxic condition, serum-free media (SFM) and serum-based media (MPRO) achieved requisite expression of positive markers (≥ 95% CD73, CD90, CD105 & CD166) and lack of expression in negative markers (≤ 5% CD14-CD19-CD34-CD45 panel & HLA-DR) with a slight increase in negative marker with SFM and MPRO.

## NSC GROWTH & IDENTITY

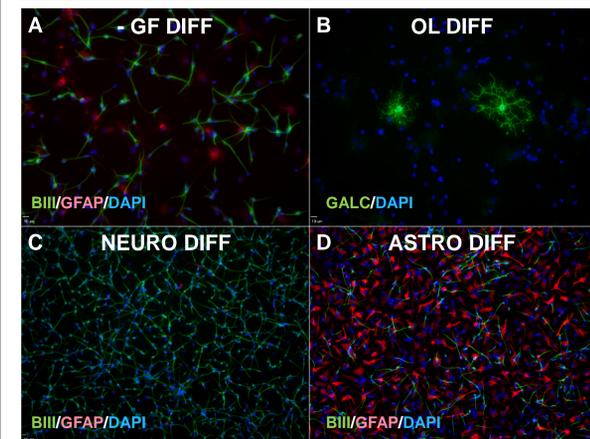


**Figure 7. Serum-Based & Serum-Free Culture Growth Kinetics & Morphological Observations**  
 ≥70% viable StemPro<sup>®</sup> NSC was plated in serum-based and serum-free media. NSC exhibited comparable growth for either type of media. After 21 days in culture, ~20 x 10<sup>6</sup> cells could be produced from an initial population of 1 x 10<sup>6</sup> viable NSC. StemPro<sup>®</sup> NSC cultured under serum-free media condition exhibited classical morphological attributes of neurospheres. These neurospheres can be passaged at least 3 times..



**Figure 8. Biomarker Analysis**  
 StemPro<sup>®</sup> NSC proliferated for 21days were plated down on slide for characterization. StemPro<sup>®</sup> NSC expressed typical neural stem cell markers of (Sox1 and Sox2) and astrocytes (GFAP). All nuclei was labeled with DAPI (blue). For Sox1 and Sox2, split channel of DAPI was resized and grayscale.

## NSC POTENCY



**Figure 9. Neural & Glial Cell Potential**  
 StemPro<sup>®</sup> NSC were plated down on slide for differentiation. After 7 days of either spontaneous or directed differentiation, neurons, astrocytes and oligodendrocytes were labeled with phenotype markers of neurons (bIII tubulin), astrocytes (GFAP) and oligodendrocytes (GALC). A) StemPro<sup>®</sup> NSC were differentiated by removal of growth factors from proliferation medium for 7 days and immunostained for neurons (green) and astrocytes (red). B-D) StemPro<sup>®</sup> NSC were differentiated to enrich either B) oligodendrocytes (GALC), C) neurons (bIII tubulin) or D) astrocytes (GFAP) for 7 days.

## SUMMARY

- StemPro<sup>®</sup> BM MSC and StemPro<sup>®</sup> NSC are able to be expanded at large scale to generate a fully characterized and standardized single source of adult stem cells manufactured in FDA compliant, GMP environment.
- StemPro<sup>®</sup> BM MSC demonstrate increased expansion rate when cultured under hypoxic condition as well as capability to be cultured in xenofree, serum-free and serum-based media under normoxic condition.
- StemPro<sup>®</sup> NSC, following similar scale-up manufacturing processes, exhibit increased growth when cultured in serum-based or serum-free media. Characterization and differentiation studies demonstrate expression of NSC biomarkers in undifferentiated NSC while neuron, oligodendrocyte and astrocyte biomarker observed in both mixed and directed differentiation.
- StemPro<sup>®</sup> BM MSC and StemPro<sup>®</sup> NSC can serve as a standardized control in cell-based assays.

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