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Comparison of human platelet lysate (HPL) and fetal bovine serum (FBS) for optimal culture conditions of neonatal and adult mesenchymal stem cells (MSCs)

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Introduction

Fetal bovine serum (FBS) has significantly contributed to the large-scale expansion of animal and human mesenchymal stem/stromal cells (MSCs) and the rapid development of cell-based therapeutics; however, it poses several regulatory and species cross-contamination challenges hindering the clinical transition of most products. Serum-free media (SFM) supplemented with several growth factors has been proposed as an alternative approach to FBS; however, custom media development is often needed based on the cell type, source, and species. Also, the high cost of SFM makes it an impractical option for large-scale cell expansion. Hence, there is a pressing need to find a human-based media additive. Human platelet lysate (HPL) is derived from human platelets and contains similar growth factors and cytokines found in FBS at comparable levels. It has been previously demonstrated that HPL supports the growth of various cells. The focus of this study was to evaluate the ability of a serum converted HPL that does not require the use of heparin (PL-NH) and standard HPL requiring heparin (PL-H) to support attachment and proliferation of neonatal and adult MSCs derived from different tissues at different concentrations of media.

Materials and methods

Variants of HPL used in this study (Stemulate™ Pooled Human Platelet Lysate NH and H) are produced at an industrial scale (minimum lot size of 20 L) with high lot-to-lot consistency and purity.

Adipose-derived, dental pulp-derived, and amniotic membrane-derived MSCs were isolated and established directly either in Stemulate or FBS immediately postisolation at 10% vol supplementation for both sera.

Adipose-derived MSCs were subsequently subcultured in DME/F-12 supplemented with 10 vol% Stemulate-NH or 10 vol% FBS for 11 passages. Amniotic-derived MSCs were cultured in DME/F-12 supplemented with 5, 7.5, or 10 vol% Stemulate-NH and compared to 10 vol% FBS. Amniotic-derived MSCs were also cultured in 2.5 or 5 vol% Stemulate-H and compared to 10 vol% FBS. Dental pulp-derived MSCs were cultured in DME/F-12 supplemented with varying concentrations of either Stemulate-NH or Stemulate-H and compared to 10 vol% FBS supplemented media. In all experiments, cells were monitored daily at each passage. At the end of each passage, cells were harvested, counted, and passaged using trypan blue and an automated cell counter.

Results

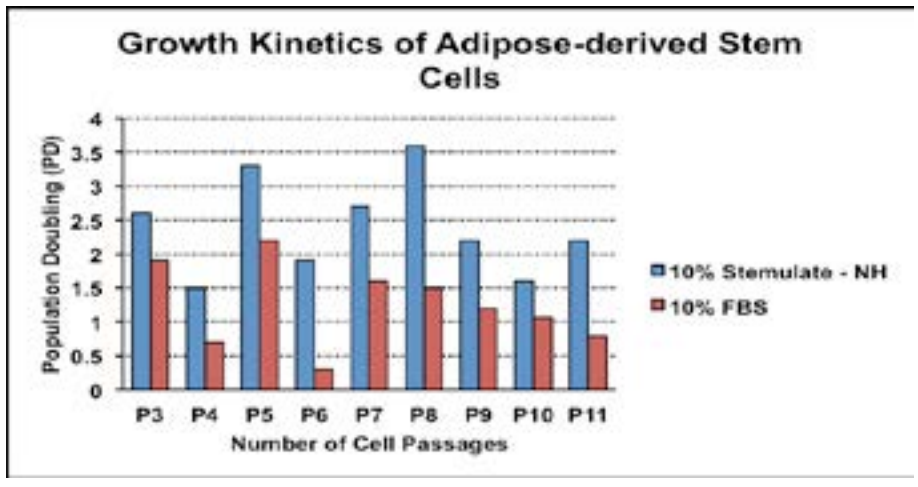

Figure 1: Growth kinetics of adipose-derived stem cells cultured in 10% Stemulate-NH and compared to 10% FBS in extended culture. Stemulate-NH does not require the addition of heparin to the media.

- Culate-NH supports isolating and establishing adipose-derived stem cells.
- Stemulate-NH supports cell proliferation throughout an extended culture period.

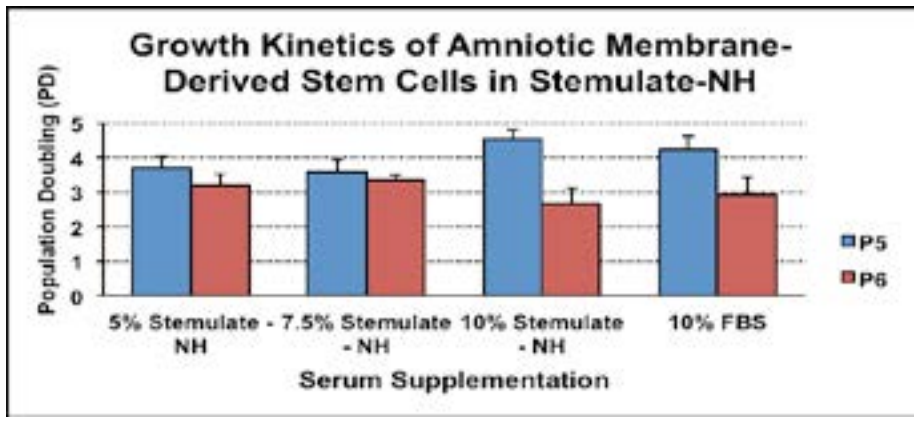

Figure 2: Amniotic membrane-derived MSCs cultured in Stemulate-NH for two passages. Stemulate-NH does not require the addition of heparin to the media.

- Stemulate-NH supports isolating and establishing amniotic membrane-derived MSCs from placental tissue.
- Low concentration of Stemulate-NH supports growth and proliferation of amniotic membrane-derived MSCs.

Results continued

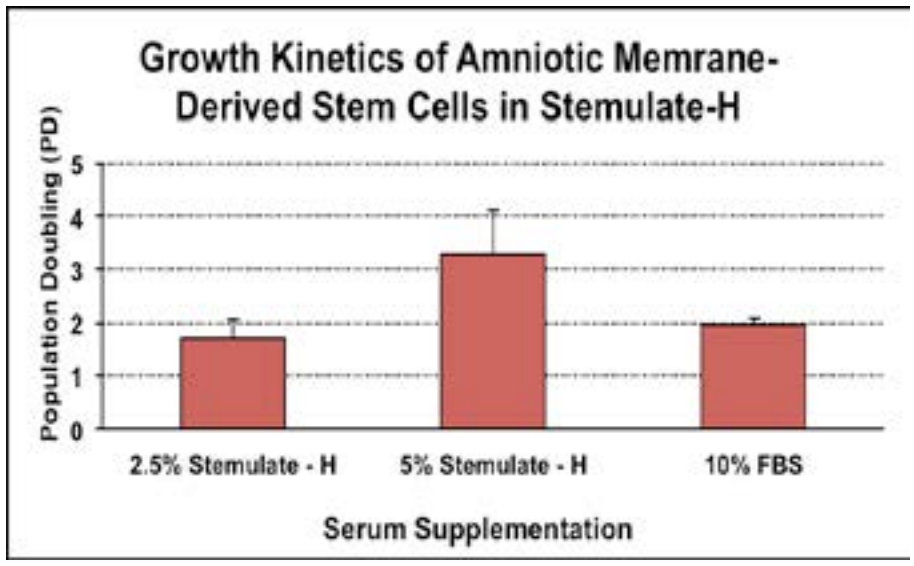

Figure 3: Amniotic membrane-derived stem cells grown in Stemulate-H at passage 5.

- Stemulate-H supports proliferation of amniotic membrane-derived stem cells in cultures for several passages.
- Low concentration of Stemulate-H supports expansion of amniotic membrane-derived stem cells.

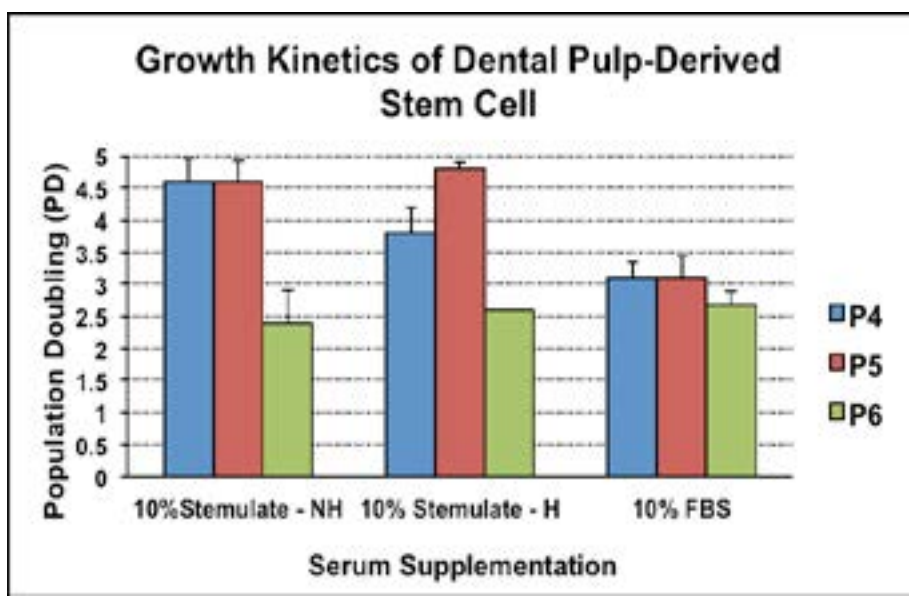

Figure 4: Proliferation of dental pulp-derived stem cells established in Stemulate-NH and expanded for several passages in Stemulate-NH or Stemulate-H.

- Both types of Stemulate (H/NH) support proliferation of dental pulp-derived stem cells for several passages.

Conclusions

- The study demonstrates the ability of both types of Stemulate (H/NH) to support isolating and expanding adult and neonatal-derived stem cells in human-based cultures.
- Stemulate is an alternative to FBS for isolating and expanding human adult and neonatal stem cells for cell-based therapeutics.
- Stemulate supports large-scale expansion of adult stem cells over extended culturing for clinical applications.