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Characterization of human platelet lysate in cytokine analysis and proliferative effect on mesenchymal stem cells

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Introduction

Human platelet lysate (HPL) has emerged as a viable human-derived alternative to fetal bovine serum (FBS) for the expansion of mesenchymal stem cells (MSCs) for clinical applications. Derived from human platelets, HPL contains similar growth factors and cytokines found in FBS at comparative or higher levels. Several methods of manufacturing HPL have been introduced, thus, HPL products differ in number of pooled donors, lot size, consistency between lots, cytokines/growth factor presence and/or levels, and heparin requirements. These differences significantly impact cell growth, morphology, stemness properties, doubling time, and senescence. The focus of this study is to characterize a new product, Stemulate™ Pooled Human Platelet Lysate, in terms of physical properties (such as turbidity and chemistry), effect on cell proliferation and morphology, profile of cytokine/growth factors, and compare it to other commercially available HPL products. Stemulate is provided in two different versions, a heparin-requiring HPL (PL-H) and heparin-free HPL (PL-NH) and is produced at an industrial scale with high lot-to-lot consistency and purity. Stemulate-H has slightly higher levels of growth factors/cytokines compared to Stemulate-NH and is comparable to HPL from other manufacturers. HPL products from different manufactures have different levels of turbidity, growth factors/cytokines, and cell proliferation and morphology. Stemulate-NH has a high level of growth factors but slightly lower than Stemulate-H. However, the performance of Stemulate-NH in cell culture is comparable to cells grown in Stemulate-H containing media. The lack of heparin requirement provides a practical xeno-free media additive and does not restrict its use for the expansion of stem cells for clinical applications of patients with special medical conditions.

Materials and methods

Two HPL products are used in this study (Stemulate NH and H) and are produced at an industrial scale (minimum lot size of 20 L) with high lot-to-lot consistency and purity. Physical properties of Stemulate are compared to several HPL products commercially available from various manufacturers.

Turbidity testing was performed using a turbidity meter, which computes the degree to which light is scattered by particles suspended in a liquid. This result is reported as nephelometric turbidity units (NTU). A NTU is a single illumination beam light source, measured at 400-680nm, 90° to the incident beam.

Growth factors were analyzed using a commercially available multiplex assay at a validated third-party testing facility.

Adipose-derived MSCs were isolated and established immediately in Stemulate. Cells were then expanded for several passages in DME/F-12 culture media supplemented with 10% vol Stemulate-NH, 10% vol for each of the two heparin-free alternatives, 5% vol Stemulate-H, and 5% vol for each of the two heparin-requiring alternatives. Each of the heparin-requiring HPLs was supplemented with 2 IU/mL of heparin. The cells were monitored at each passage daily. At the end of each passage, cells were counted using a trypan exclusion method and passaged.

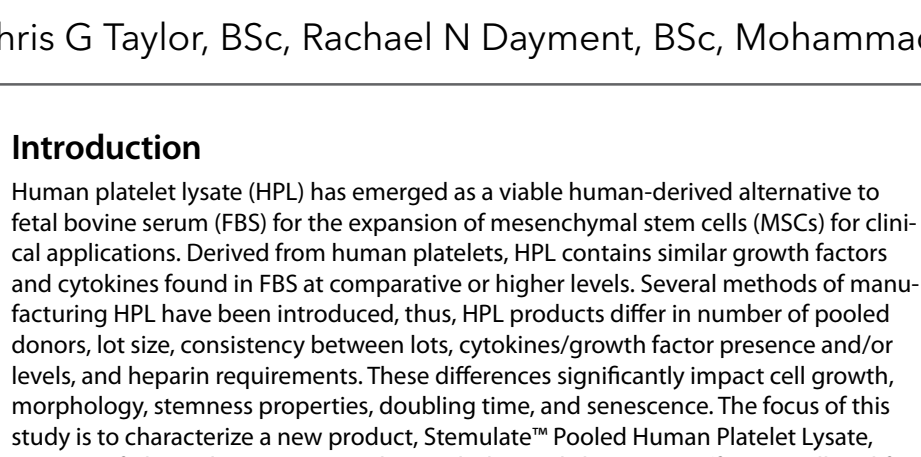

Figure 1: Turbidity of Stemulate-H and two heparin-requiring HPL products.
*Denotes p<0.05 compared to Stemulate-H

- Stemulate-H has very low turbidity appearance compared to that of other heparin-requiring HPL serums.

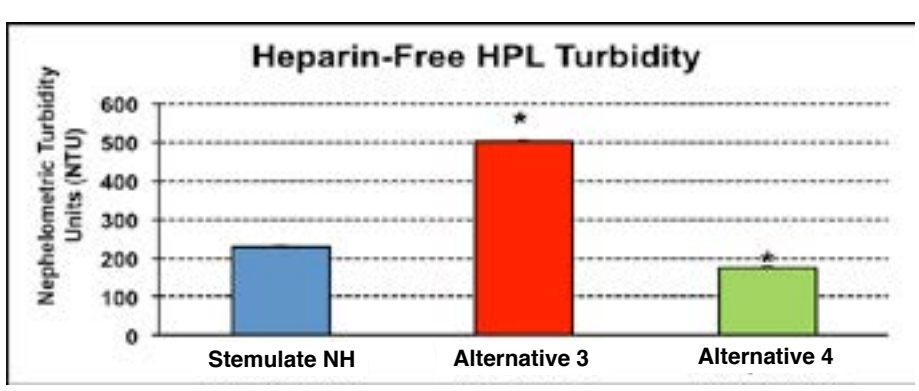

Figure 2: Turbidity of Stemulate-NH and two heparin-free HPL products.
*Denotes p<0.05 compared to Stemulate-NH

- Stemulate-NH has very low turbidity appearance compared to other heparin-free HPL serums.

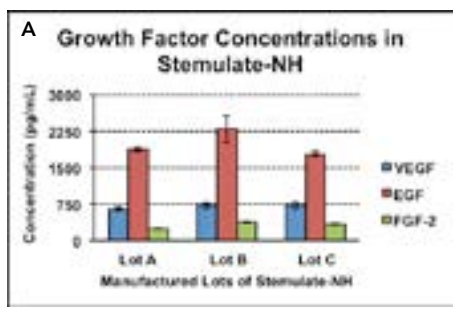

Figure 3a: Growth factor profile of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor 2 (FGF-2) across multiple lots of Stemulate-NH.

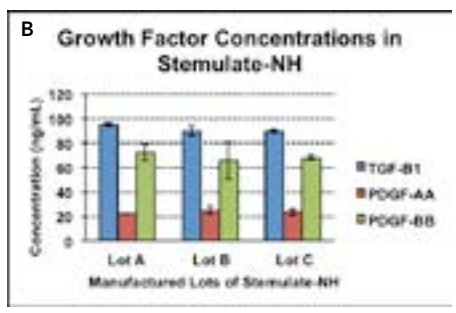

Figure 3b: Growth factor profile of transforming growth factor ß1 (TGF-ß1), platelet-derived growth factor AA (PDGF-AA) and platelet-derived growth factor BB (PDGF-BB) across multiple lots of Stemulate-NH.

- Stemulate-NH has a consistent growth factor profile across multiple manufactured lots.
- Stemulate-NH has high levels of essential growth factors necessary for cell growth and angiogenesis.

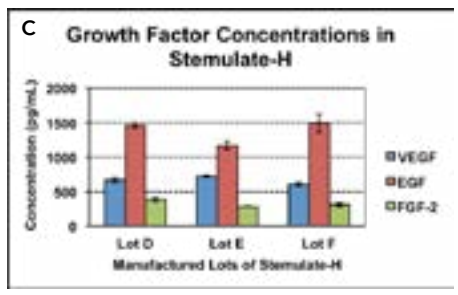

Figure 3c: Growth factor profile of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor 2 (FGF-2) across multiple lots of Stemulate-H.

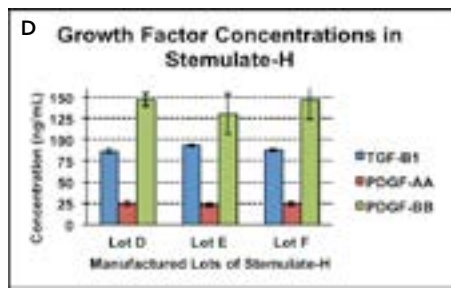

Figure 3d: Growth factor profile of transforming growth factor ß1 (TGF-ß1), platelet-derived growth factor AA (PDGF-AA) and platelet-derived growth factor BB (PDGF-BB) across multiple lots of Stemulate-H.

- Stemulate-H has a consistent growth factor profile across multiple manufactured lots.
- Stemulate-H has substantial amounts of growth factors crucial for cell proliferation and biological activities including angiogenesis.
- Stemulate-H has higher levels of VEGF, FGF-2, and PDGF-AA/BB compared to Stemulate-NH.

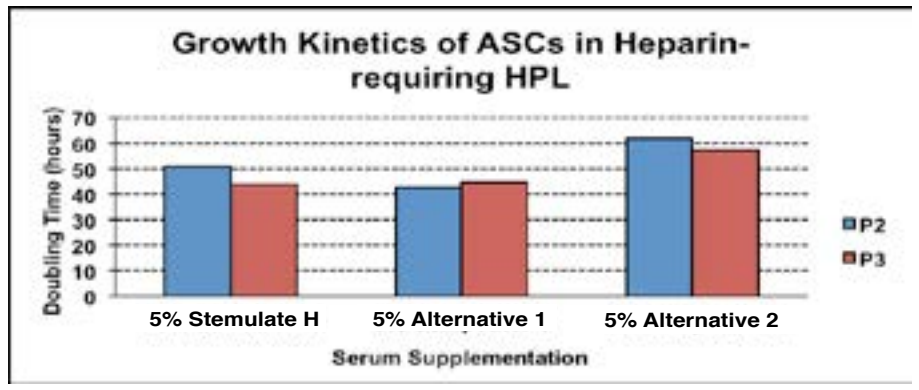

Figure 4: ASCs grown in Stemulate-H and other heparin-requiring HPL at passage 2 and 3.

- Stemulate-H supports proliferation of ASCs in cultures for multiple passages.
- Expansion of ASCs in Stemulate-H is at comparable levels to that of heparin- requiring HPL products.

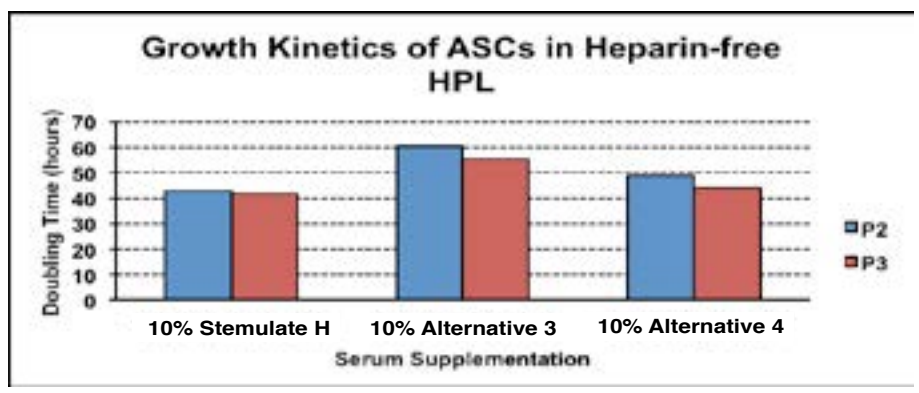

Figure 4: ASCs grown in Stemulate-NH and other heparin-free HPL products at passage 2 and 3

- Stemulate-NH supports proliferation of ASCs in cultures for multiple passages.
- Expansion of ASCs in Stemulate-NH is at comparable levels to that of heparin-free HPL products.

Conclusions

- Stemulate has high levels of essential growth factors for cellular growth and functions.
- The study demonstrates the lot-to-lot consistency of both types of Stemulate H/NH in terms of growth factor profile.
- Stemulate can substitute for FBS in the isolation and expansion of human adult and neonatal stem cells for cell-based therapeutics.

PI-BP-SCIPOSK-EN-201404