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Human platelet lysate supports growth of human skin and vascular-derived cells similar to fetal bovine serum and serum-free media

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Introduction

Acellular extracellular matrices (ECM) such as dermis and blood vessels have advanced the field of regenerative medicine and have been used in life-saving clinical practices. However, the prolonged angiogenesis and homing of endogenous cells impede tissue remodeling and may lead to graft failure. Dermal fibroblasts, keratinocytes, and vascular endothelial cells are commonly used for research. However, these cells are often grown in FBS supplemented media, which hinders their rapid transition into clinical application in the field of skin and vascular tissue engineering due to regulatory challenges and risk of disease transmittance. Thus, there is a 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 demonstrated that hPL supports the growth of various cells. The focus of this study was to evaluate the ability of heparin-free HPL (PL-NH) and HPL requiring heparin (PL-H) to support proliferation and maintenance of primary human cells from different tissues at different concentrations of media. Stemulate™ Pooled Human Platelet Lysate is produced at an industrial scale (minimum lot size 20 L) with high lot-to-lot consistency and purity.

Materials and methods

Foreskin samples from different donors were obtained from circumcision procedures. Dermal fibroblasts were obtained by tissue digestion or explant culture protocols. Fibroblasts were established in culture media supplemented with 10% vol Stemulate-NH or FBS immediately post-isolation. Cells were then grown in different concentrations of Stemulate-NH and compared to cells grown in FBS over several passages.

Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza and were established in endothelial growth media (EGM-2) supplemented with a bullet kit containing 2% FBS. HUVECs were weaned in 10% Stemulate for two passages before being used in the experiment. HUVECs were then grown in different concentrations of both types of Stemulate replacing the FBS in the bullet kit.

Results

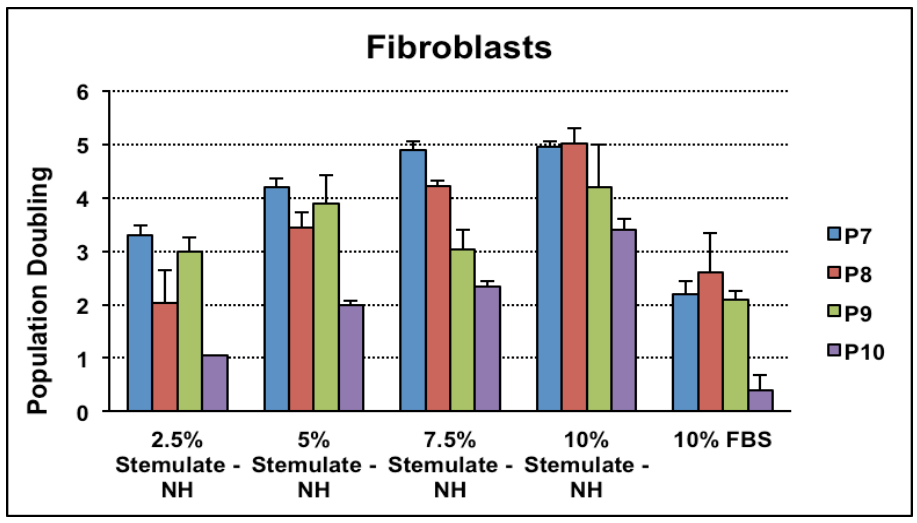

Figure 1: Proliferation of dermal fibroblasts grown in Stemulate-NH and FBS over multiple passages. Stemulate-NH does not require the addition of heparin to the media.

- Stemulate-NH supports the isolation and expansion of dermal fibroblasts from human foreskin.
- Low concentrations of Stemulate support proliferation of fibroblasts for several passages.

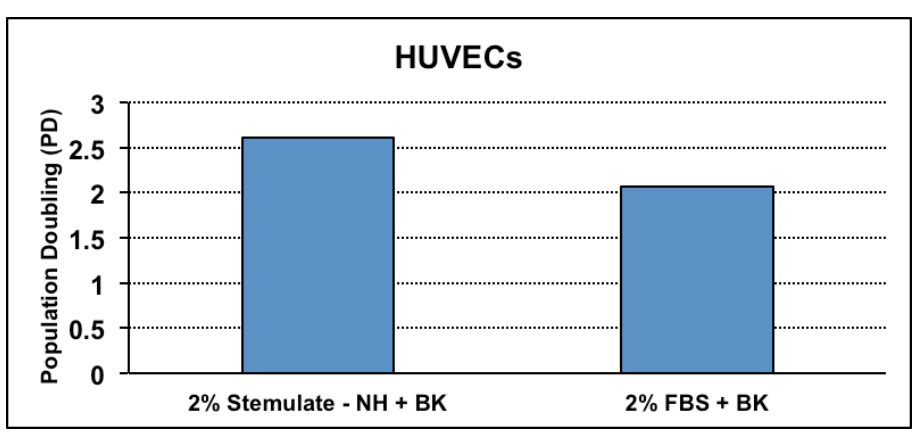

Figure 2: Growth of HUVECs when FBS is replaced with Stemulate-NH in the bullet kit. Stemulate-NH does not require the addition of heparin to the media.

- Stemulate-NH supports the growth and proliferation of HUVECs.
- Stemulate-NH can replace FBS from EGM-bullet kit for xeno-free culture of HUVECs.

Results continued

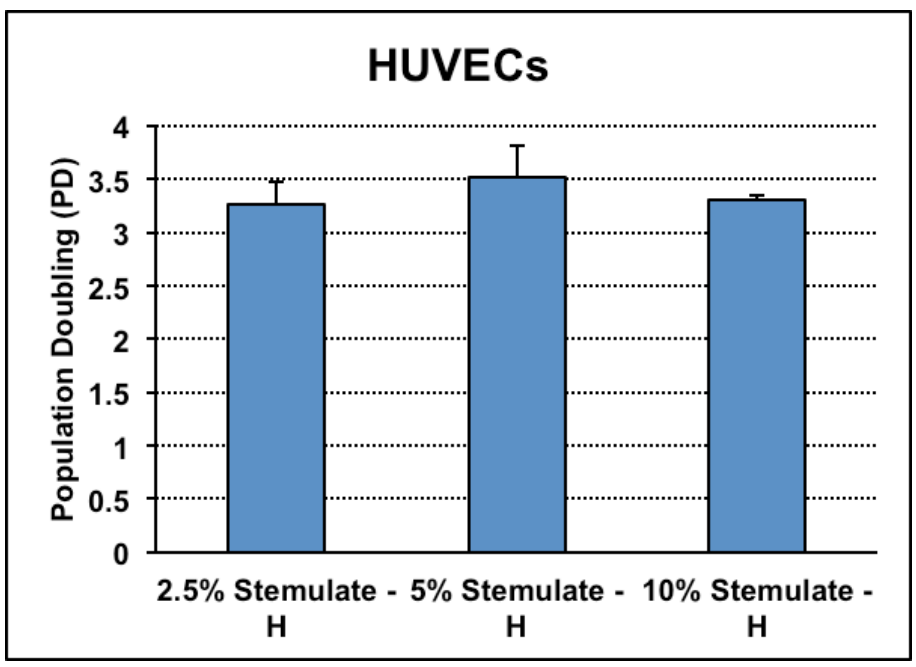

Figure 3: HUVECs grown in different concentrations of Stemulate-H after 14 doublings. Stemulate-H requires the addition of heparin to the media.

- Stemulate-H supports the proliferation of HUVECs.
- Low concentrations of Stemulate support the proliferation of HUVECs after 14 doublings in culture.

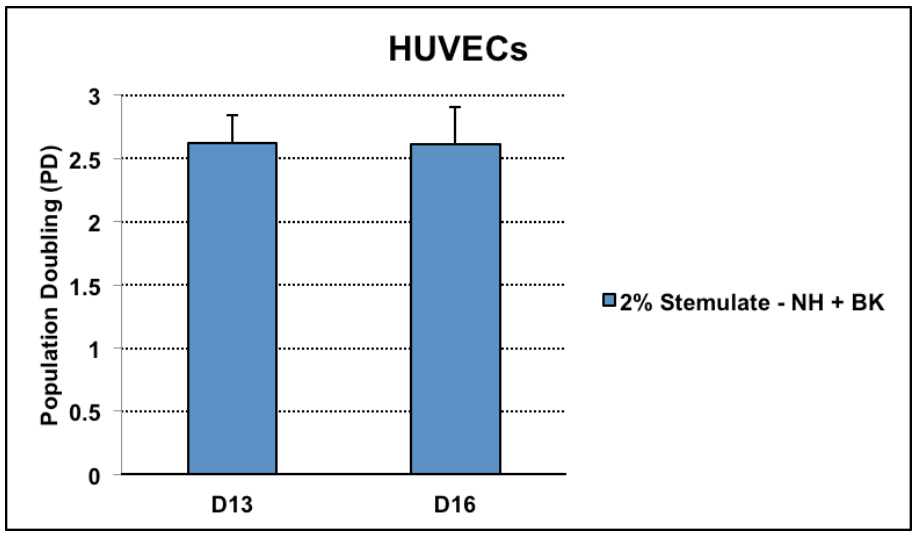

Figure 4: HUVECs at different doublings grown in low concentration of Stemulate-NH. Stemulate-NH does not require the addition of heparin to the media.

- Low concentrations of Stemulate-NH support and maintain proliferation rates of HUVECs after several doublings in culture.

Conclusions

- Low concentrations of Stemulate support the growth and proliferation of fibroblasts and HUVECs over several passages in cultures.
- Stemulate can replace FBS for the expansion of dermal fibroblasts and HUVECs for clinical applications.

PI-BP-SCIPOSF-EN-201404