Human Platelet Lysate (HPL) is Optimal for Expansion of First Trimester Human Umbilical Cord Perivascular Cells for Regenerative Medicine Applications

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INTRODUCTION

Mesenchymal stromal cells (MSCs) isolated from various sources including bone marrow, adipose tissue and umbilical cords have received much attention for their potential in regenerative medicine and cellular therapies. MSC expansion protocols typically include fetal bovine serum (FBS) to supplement culture media. FBS is collected from fetal cows at typically 6 months of gestation, were serum is separated from clotted blood (Figure 1A) (1). Limitations of FBS include, lack of defined components, lot-to-lot variability and safety concerns.

In order to follow good manufacturing practice (GMP), the transition to xeno-free media to culture MSCs is required for clinical practice. Human platelet lysate (HPL) is a commercially manufactured supplement serving as a cytokine rich replacement for FBS. HPL is derived from platelets obtained from FDA-registered blood banks. Platelet membranes are disrupted via repeated freeze-thaw cycles that initiate the release of various cytokines and growth factors (Figure 1B) (1). Advantages of HPL includes wide cell-type applicability, enriched with growth factors to support cell proliferation, easily obtained and has limited lot-to-lot variability with increasing donors. Limitations include, not being precisely defined and possible safety concerns.

In our lab we study a young source of MSC derived from first trimester umbilical cords (FTM HUCPVCs). Our lab is investigating the cellular therapeutic potential of FTM HUCPVCs in many applications including cardiovascular and neurovascular diseases. For our pre-clinical studies, GMP of FTM HUCPVCs expansion needs to be established. We obtained HPL from Compass Biomedical PLUS™ Human Platelet Lysate (2) and FBS from GE Healthcare Sciences. We aimed to compare phenotypic, homing and angiogenic profiles of FTM HUCPVCs expanded in FBS vs. HPL.

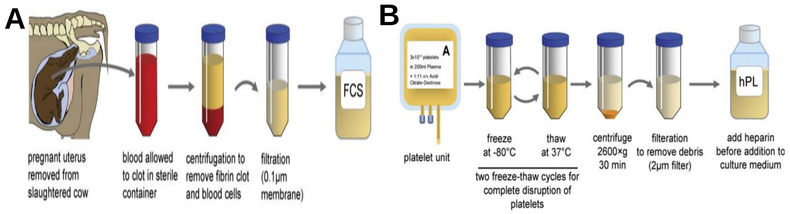
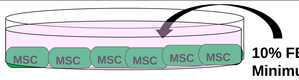


Figure1 : Generation of fetal bovine serum (A) and human platelet lysate (B).

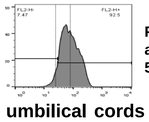
OBJECTIVES

1. **To characterize FTM HUCPVCs following *in vitro* expansion by flow cytometry**
2. **To study FTM HUCPVC homing and endothelial integration using the Aortic Ring Assay (3)**
3. **To characterize the gene expression of angiogenic growth factors of FTM HUCPVC following co-culturing with Aortic Ring Assay**
4. **To quantify FTM HUCPVC-mediated recruitment of mouse vasculature and neo-angiogenesis of injected Matrigel™ plugs**

METHODS: Immunophenotyping



10% FBS or 5% HPL with α-Minimum Essential Medium Eagle



Flow Cytometry analysis at passage 3, 5, 7, 9

Figure2: Expansion of primary FTM HUCPVCs from umbilical cords (week8-10) in FBS or HPL supplemented media. Flow cytometry analysis carried out passage 3, 5, 7 and 9.

METHODS: Angiogenesis Potency Assays

In vitro Angiogenesis Assay: Aortic Ring Assay

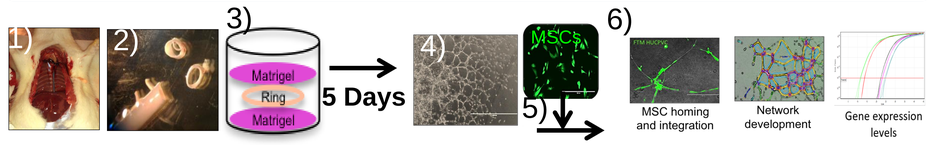


Figure3: Set-up of the aortic ring assay[3]: 1) Aorta Isolation 2) Sectioning of aorta 3) Embedding of aortic rings between Matrigel 4) Development of endothelial networks (5 Days) 5) Pre-stained MSCs co-cultured with developing endothelial networks 6) Assessment of MSC migration, integration, overall effect on network development and changes in gene expression.

In vivo Angiogenesis Assay: Matrigel Plug Assay

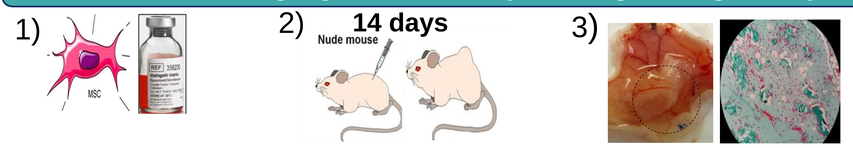


Figure4: Set-up of Matrigel Plug Assay: 1) Suspension of MSCs with Matrigel 2) Subcutaneous co-injection of MSCs and Matrigel in nude mice for 14 days 3) Isolation of Matrigel plugs and quantification of host vasculature recruitment and quantification of new blood vessels in Matrigel plugs following Masson's trichrome staining.

RESULTS

FTM HUCPVCs Expanded in 5%HPL Exhibit Similar Immunophenotypical Profiles When Expanded in Standard 10%FBS Conditions at Passage 5

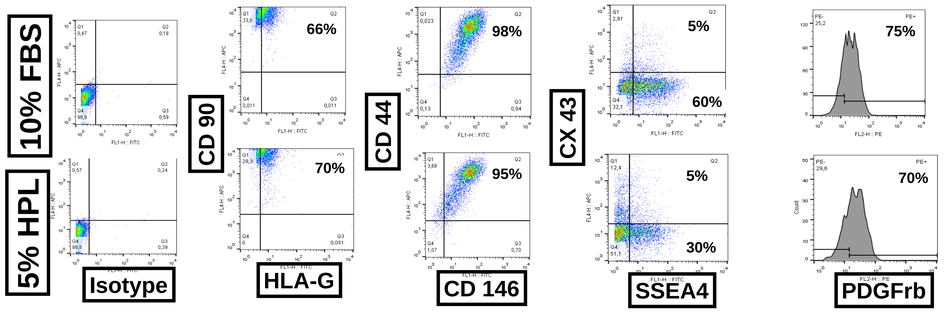


Figure5: Immunophenotypical analysis plots of FTM HUCPVCs at passage 5 suggest strong and similar MSC and pericyte phenotypes when expanded in FBS and HPL.

FTM HUCPVCs in Both FBS and HPL Home to Endothelial Networks and Contributed to Similar Endothelial Network Development

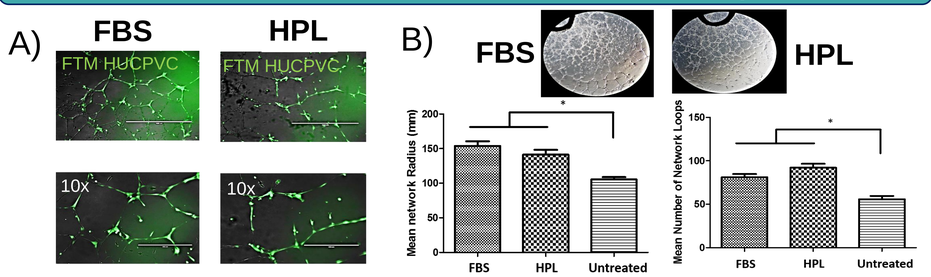
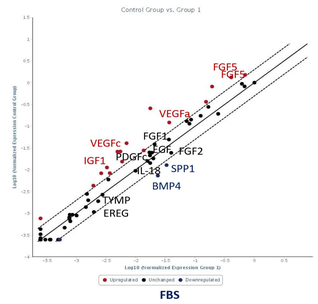


Figure6: A) FTM HUCPVCs homed to peripheral developing endothelial networks. High magnification images show elongated morphologies and a continuous network with the formation of closed loops in both conditions. B) Microscopic images of FTM HUCPVC cultured with endothelial networks resulted in greater network growth and loop formation when compared to untreated networks (*P<0.05)* in both conditions.

RESULTS

Most Angiogenic Growth Factors are Similarly Expressed by FTM HUCPVCs expanded in FBS and HPL Following 1 week culture in Aortic Ring Assay

A)



B)

Growth factor	Ct Value (FBS)	Ct Value (HPL)	Fold Change
TGFB1	22	18	13
VEGFA	23	21	3
VEGFC	22	25	-7
FGF1	25	24	-
FGF2	22	22	-
FGF5	23	21	3
PGF	30	27	7
PDGFC	23	22	-
EGF	23	25	-5
SPP1	27	22	44
TYMP	26	24	6
TNNT1	30	27	5
EREG	29	27	5

Figure7: Evaluation of FTM HUCPVC gene expression: angiogenic factors following direct co-culture with aortic rings. A) Most angiogenic factors are highly expressed by both FTM HUCPVCs expanded in FBS and HPL (Black). VEGF and FGF family factors are upregulated in HPL expanded FTM HUCPVCs (Red). B) Fold changes are described in table. All factors highlighted in figure A have ct<30

Similar Vasculature Recruitment and Development of Perfused Blood Vessels in Matrigel Plugs Treated With FTM HUCPVCs Expanded in FBS and HPL

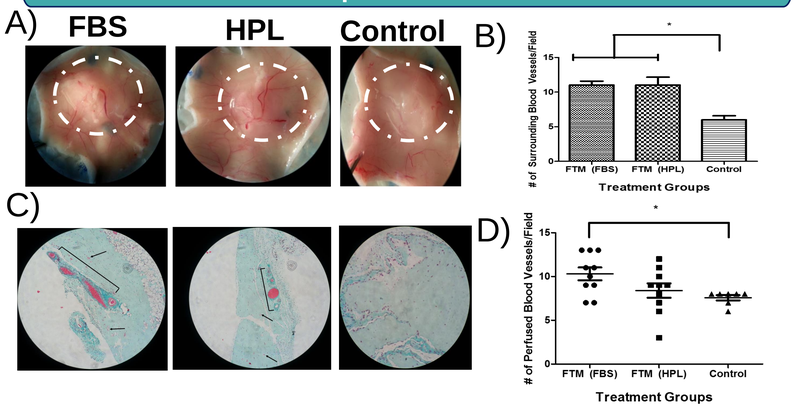


Figure8: Angiogenic response in Matrigel plugs 14 days post- implantation: A) B) Microscopic images of Matrigel Plugs injected with FTM HUCPVCs . C) D) There was no significant difference (p>0.05) between FTM HUCPVCs expanded in FBS and HPL in terms of vasculature recruitment to Matrigel plugs and development of perfused blood vessels within Matrigel Plugs. Statistical analysis was conducted using One-Way Anova N=3

CONCLUSIONS

- **FTM HUCPVCs expanded in 5% HPL exhibit similar immunophenotypical profiles, homing and angiogenic potential, both *in vitro* and *in vivo*, when compared to expansion under standard 10% FBS conditions.**
- **Our data provides a strong foundation for the development of serum-free/xeno-free GMP-compatible conditions to expand FTM HUCPVCs for regenerative medicine applications.**

ACKNOWLEDGMENTS

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