Comparison Of Fetal Bovine Serum And PLTMax® For Long Term Maintenance Of Human Induced Pluripotent Stem Cell Derived Retinal Pigment Epithelial Cells

Jarel K Gandhi, Travis Knudsen, Matthew Hill, Lori Bachman, Alan D Marmorstein

Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota

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Gandhi.Jarel@mayo.edu

PURPOSE

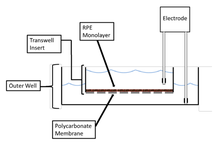
- **To evaluate non-xenogeneic media components to replace fetal bovine serum (FBS) during the maintenance of induced pluripotent stem cell retinal pigment epithelium (iPSC-RPE).**

INTRODUCTION

- iPSC-RPE are differentiated using a defined media and maintenance media is often supplemented with FBS to reduce cost.
- However, FBS has significant lot-to-lot variability and is not compliant with cell manufacturing for clinical use.
- PLTMax® (PLTM) is a human platelet lysate used as a replacement for FBS in manufacturing human mesenchymal stem cells (MSC) for clinical use [1].
- PLTGold® (PLTG) is a similar human platelet lysate that is formulated without a heparin supplement and does not result in fibrin precipitate formation in the media.
- **Therefore, we set out to evaluate PLTM and PLTG to replace FBS in maintenance media for iPSC-RPE.**

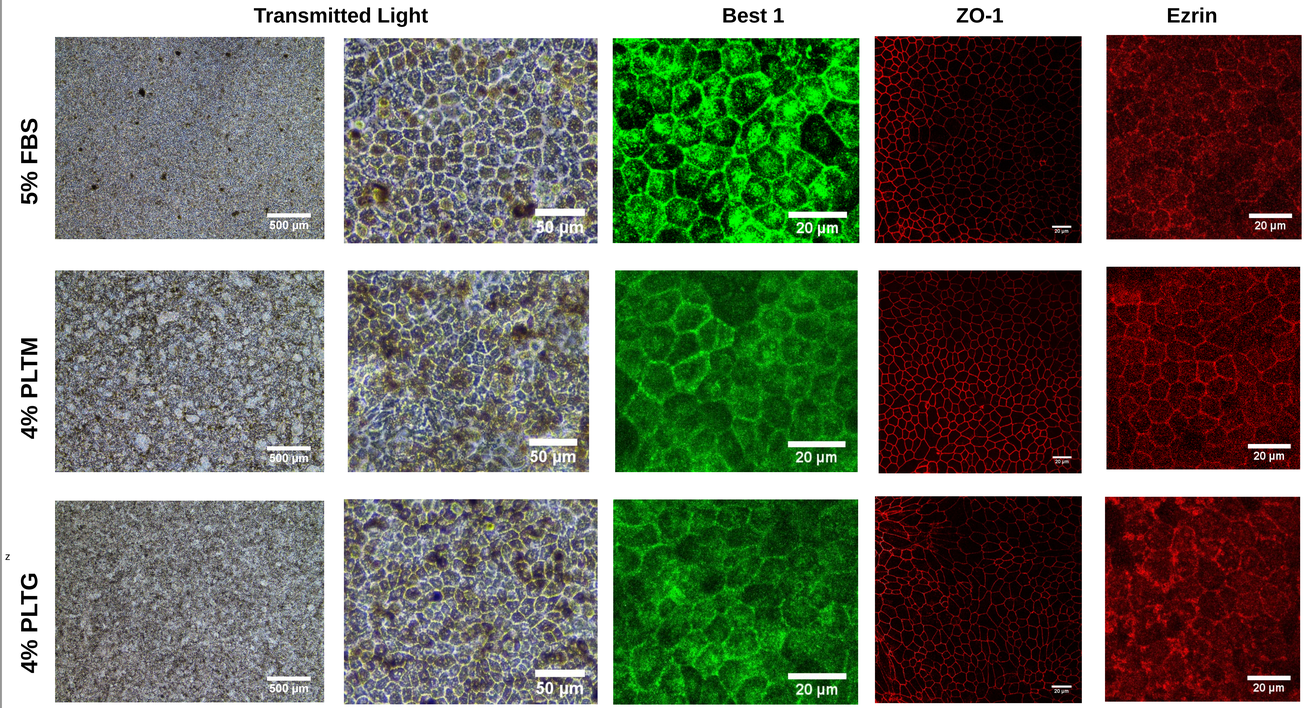
METHODS

- IPSC-RPE cells were differentiated, passaged and plated onto matrigel-coated plates or 12-well transwell as described previously [2, 3].
- Passaged RPE were cultured in RPE medium (RPEM, LAgen Labs) supplemented with B27 Supplement (ThermoFisher) for an initial 30 days, with media changes every 2 days. The media was then switched to RPEM supplemented with 5% FBS (ThermoFisher), 4% PLTM (Mill Creek), or 4% PLTG (Mill Creek).
- Immunofluorescent (IF) staining was performed with anti-Best1, ZO-1, and Ezrin antibodies as previously described [3].
- Trans Epithelial Resistance (TER) measurements were performing using EVOM2 with STX2 electrode (WPI) in 12 well Transwell plates as previously described [4], starting with Day 0 of media switch.



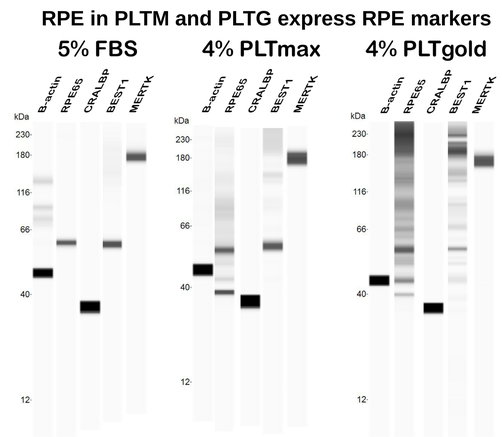
- PEDF expression was quantified using an ELISA kit (R&D Systems).
- Western Blot analysis was performed using ProteinSimple Wes [3].

RESULTS



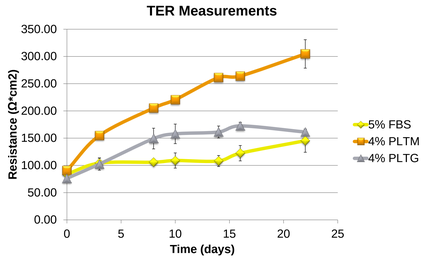
- iPSC-RPE appear pigmented cells with characteristic cobblestone appearance in all 3 media supplements. The phenotype is observed as early as 3 weeks.
- IF staining of Best1 appears basolaterally in all 3 media supplements, with patches of positively stained cells throughout the monolayer.
- IF staining of ZO1 appears strong along the lateral edges of the cells in all 3 media supplements.
- IF staining of Ezrin appears punctate along the apical surface of the cells in all 3 media supplements.

RPE in PLTM and PLTG express RPE markers



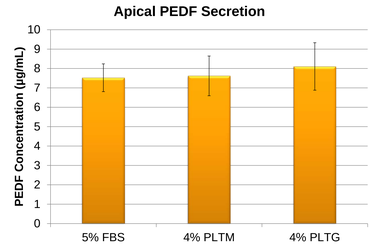
All iPSC-RPE expressed RPE markers RPE65, CRALBP, Best1, and MERTK.

RPE in PLTM, PLTG, and FBS exhibit increasing TER over time



All iPSC-RPE showed increasing TER measurements over time. iPSC-RPE cultured with PLTmax was the highest of the 3 conditions.

RPE in PLTM and PLTG release apical PEDF



iPSC-RPE release similar amounts of apical PEDF in all 3 media conditions.

CONCLUSIONS

- iPSC-RPE maintain expression of RPE markers and polarity when cultured with PLTMax and PLTGold
- TERs of iPSC-RPE cultured in PLTMax and PLTGold are equal to or superior to those cultured in FBS
- PLTMax and PLTGold are preferable media supplements to FBS for iPSC-RPE maintenance that are compatible with cell manufacturing for clinical trials

REFERENCES

1. Guess AJ et al, Stem Cells Transl Med, 6: 1868-1879, 2017.
2. Johnson A et al., Invest Ophthalmol Vis Sci., 56: 4619-4630, 2015.
3. Gandhi JK et al., Acta Biomater., 67:134-146, 2018.
4. Brandl C et al. Neuromolecular Med., 16: 551-564, 2014.

SUPPORT

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DISCLOSURE

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