Platelet Lysate Performance in Endothelial Cell Culture

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Dec. 2, 2023

Background Information

Goal

- Assess the performance of Platelet Lysate as a replacement for FBS in our EC cultures. Assess the EC's ability to grow and maintain proper phenotype in 2D and the ability of the ECs to form and maintain stable vasculature in 3D.

Materials

- EGM-2 Medium (Endothelial Cell Growth Medium 2 – PromoCell) is used to expand our ECs. This media contains 2% FBS when made according to manufacturer's directions.
  - EGM-2 can be purchased with a supplement mix (contains all growth factors and FBS) **or** as a supplement pack (each growth factor and FBS are provided individually). The supplement pack is used for these experiments.
- HUVECs were purchased from Lonza
  - Transduced with an EFS-tdTomato lentivirus for easy monitoring of ECs
- Porcine Gelatin Type A from Sigma-Aldrich is diluted in DI H_2O to form a 0.2% Gelatin solution. This solution is used as a coating for TC plastics during 2D EC expansion
- Bovine Fibrinogen from Sigma-Aldrich is diluted in EGM-2 to make a 12mg/mL fibrinogen solution.
- Thrombin from Sigma-Aldrich is diluted in PBS++ to make a 6U/mL thrombin solution.
- AIM Biotech idenTx microfluidic devices (MFDs) were used during this pilot study.

Experimental Set-Up

Media Preparation

- EGM-2 (Cat #39211) was prepared by mixing all individual components of the supplement pack with basal medium, then adding PenStrep to a final concentration of 1% in the total media volume.
- The EGM-2 solution was then split into multiple aliquots that received either 2% FBS (control), 2% Platelet Lysate, 5% Platelet Lysate, or 10% Platelet Lysate.
    - Serum added to 25mL of EGM-2 (containing growth factors+PenStrep) for each condition:
        - 500µL FBS or Platelet Lysate for 2% condition
        - 1.25mL Platelet Lysate for 5% condition
        - 2.5mL Platelet Lysate for 10% condition

2D Procedures

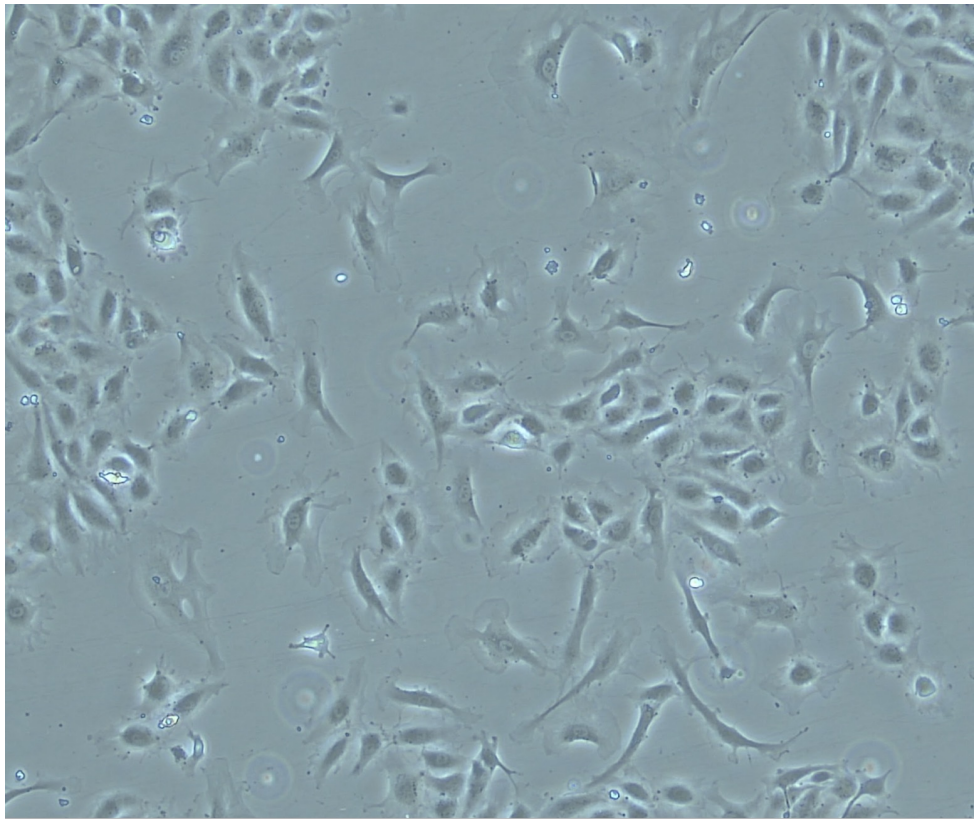
- 250K HUVECs were thawed into a T25 coated with 0.2% Gelatin and fed every other day until 80-90% confluent, at which point they were collected for 3D experiments

3D Procedures

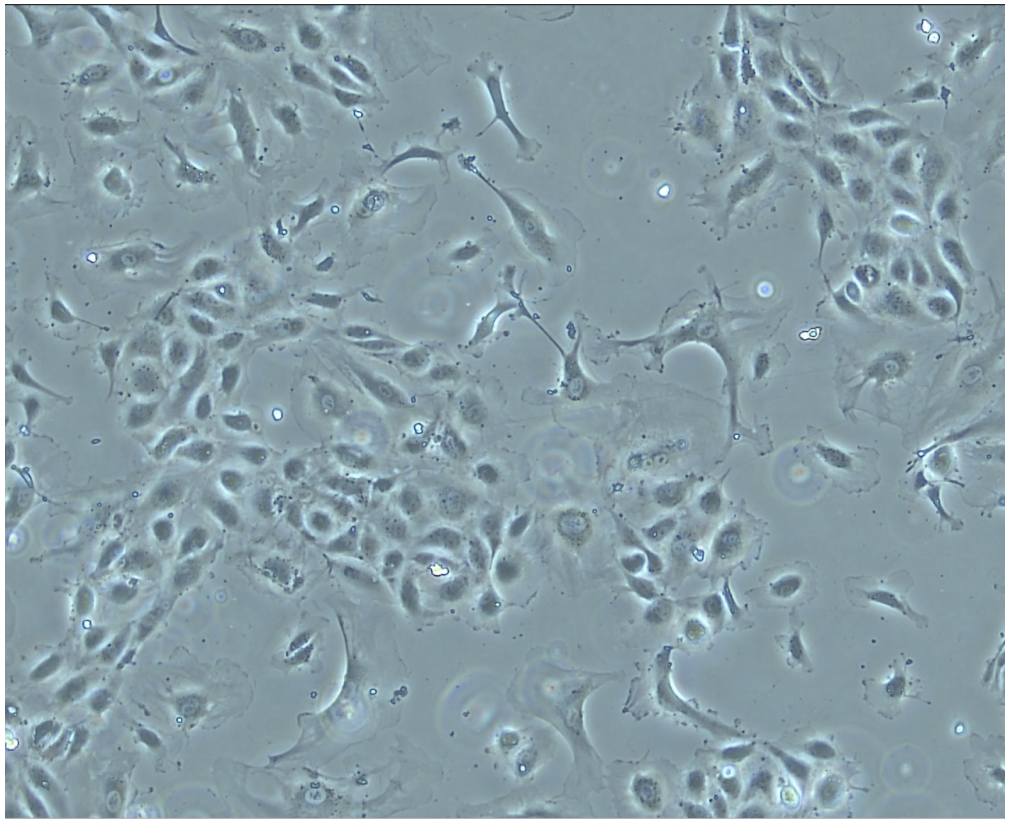
- HUVECs and Fibroblasts were resuspended in a fibrinogen solution. The fibrinogen+cells solution is mixed in a 1:1 volume ratio with thrombin to form a fibrin hydrogel containing cells. The final fibrin concentration in this solution is 6mg/mL fibrinogen + 3U/mL thrombin and the final cell ratios are 6mil ECs: 2mil Fibroblasts.
- Microfluidic devices were fed daily under interstitial flow and fibrin dot controls were also fed daily.

10% Platelet lysate is comparable to 2% FBS in 2D (4X)

2% P.L. (DAY 7)



5% P.L. (DAY 7)



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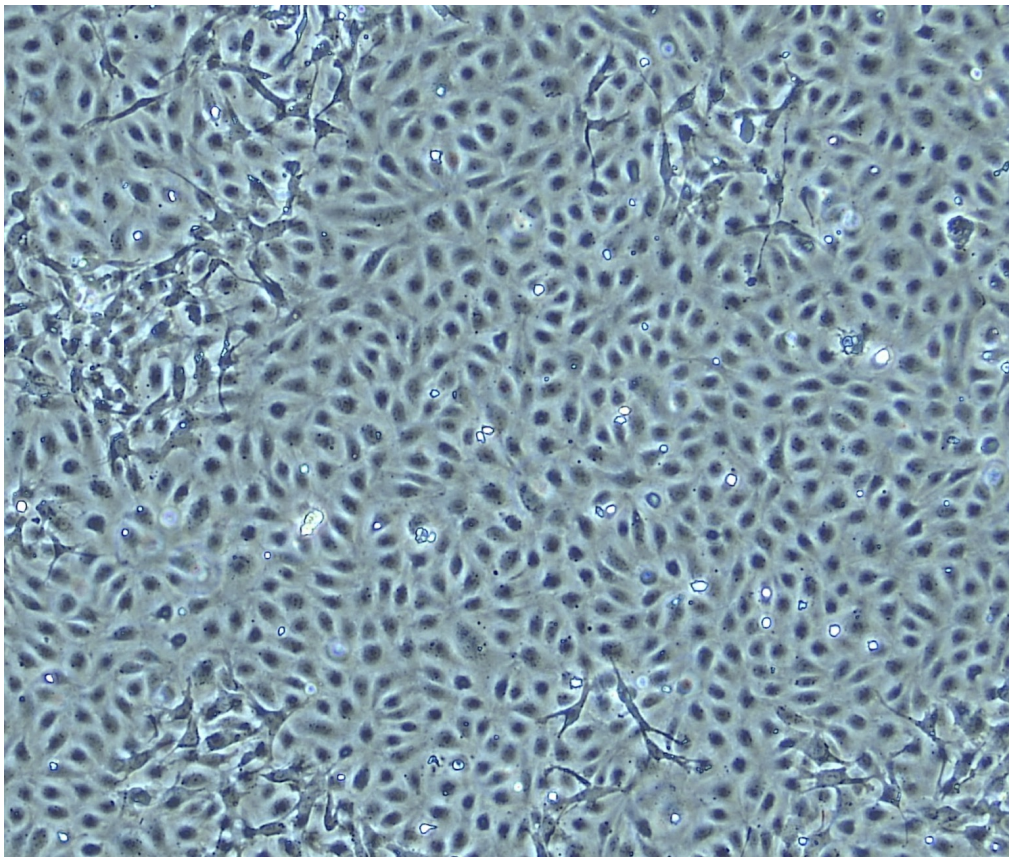
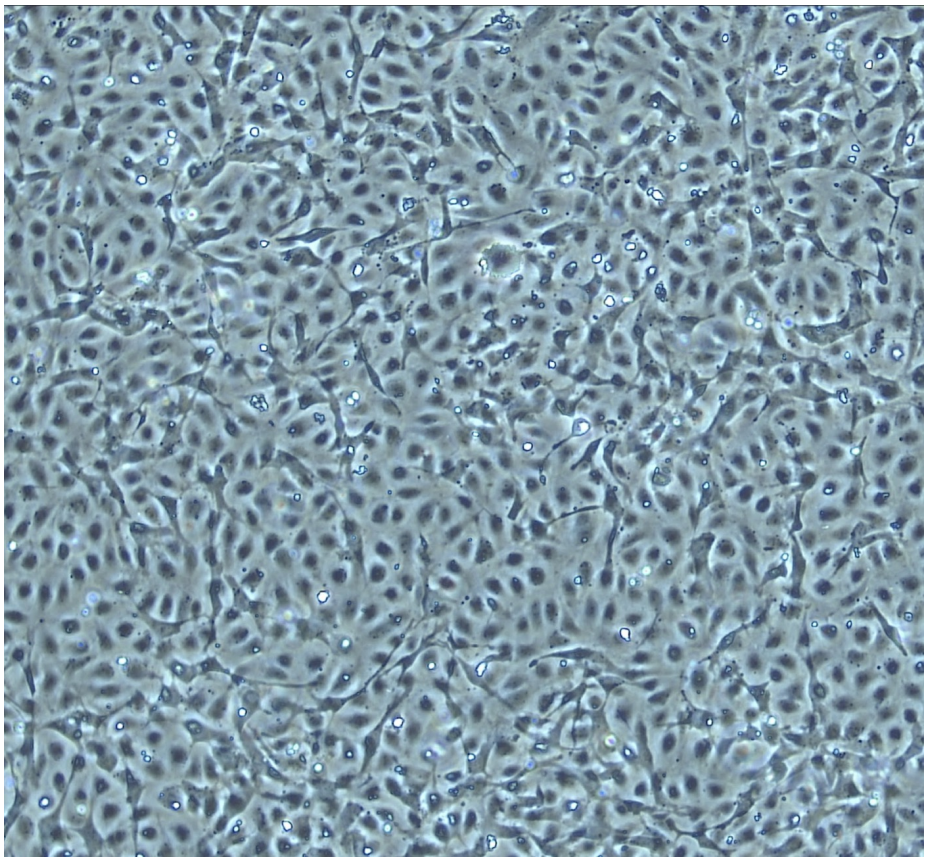


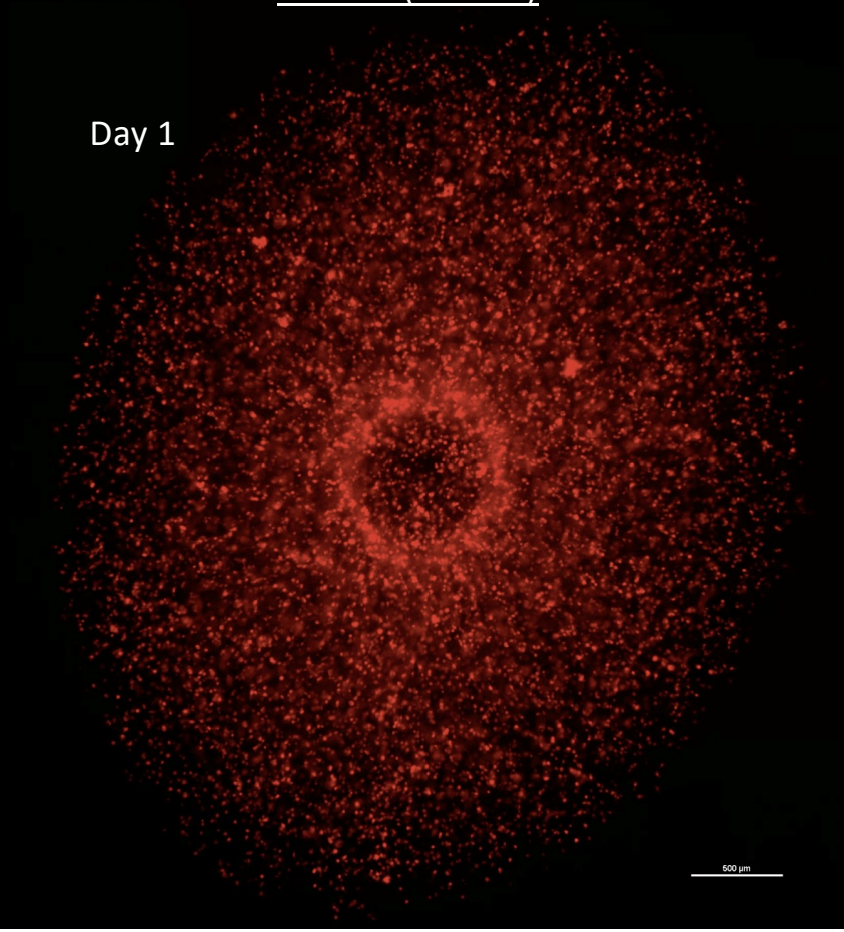
2% FBS (DAY 7)



10% P.L. promotes vascular formation in 3D fibrin dots

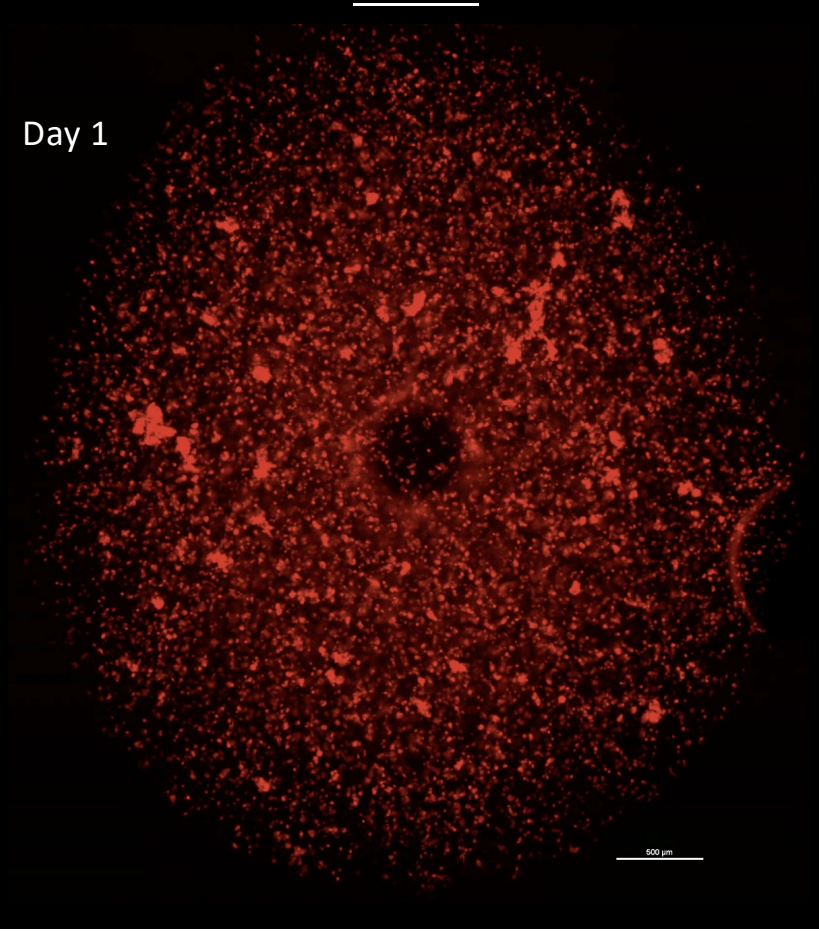
Control (2% FBS)

Day 1



10% P.L.

Day 1



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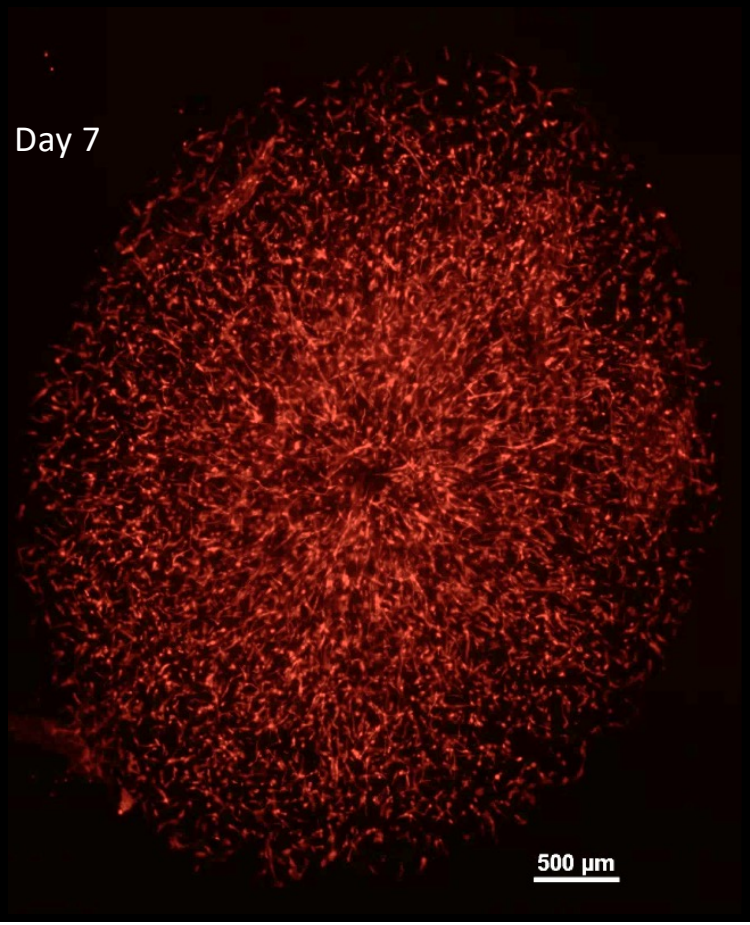
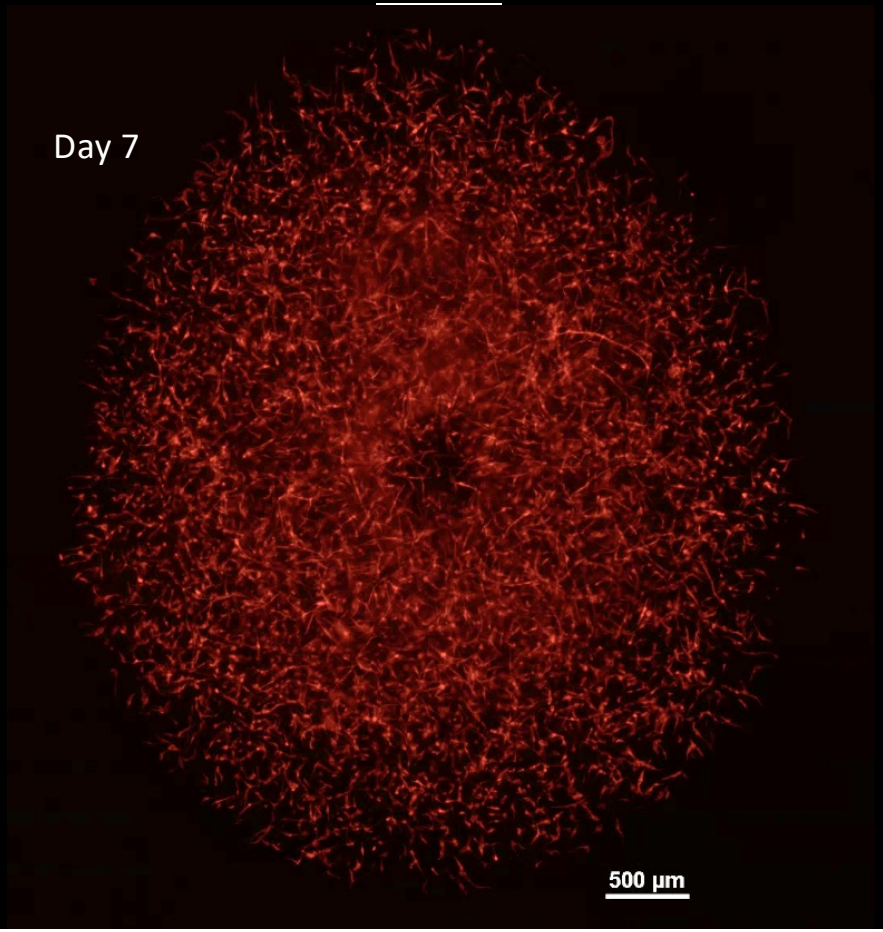


10% P.L.



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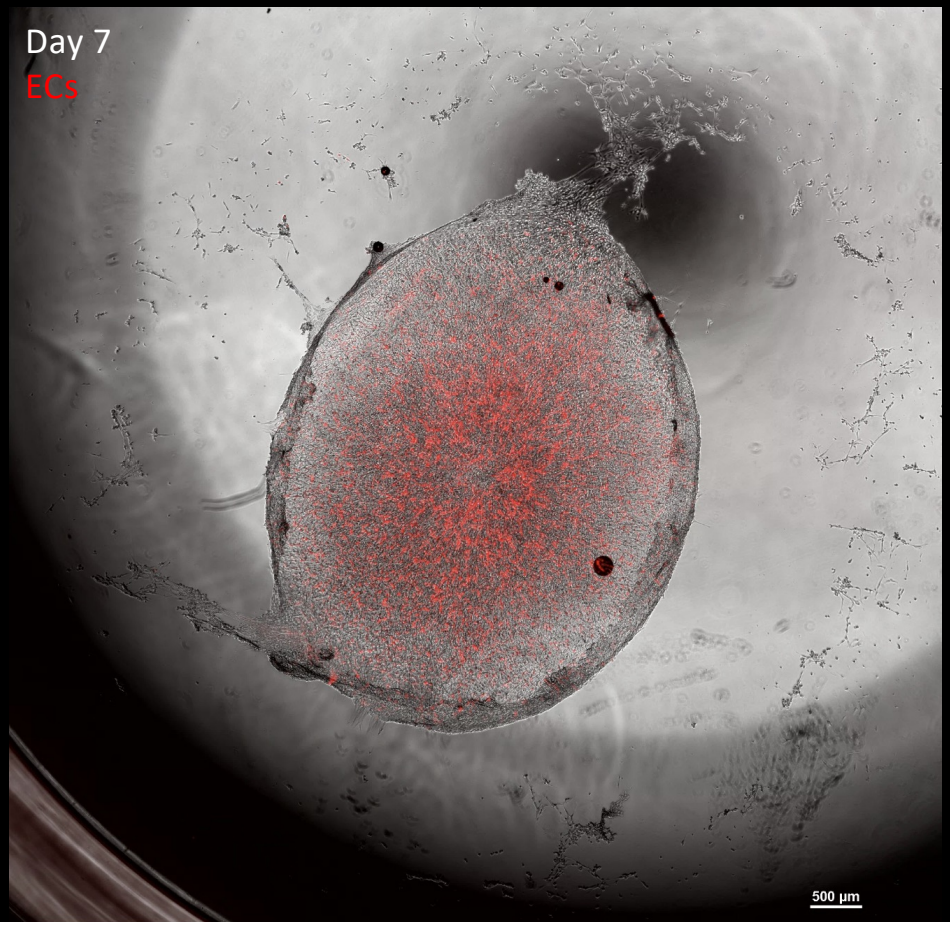
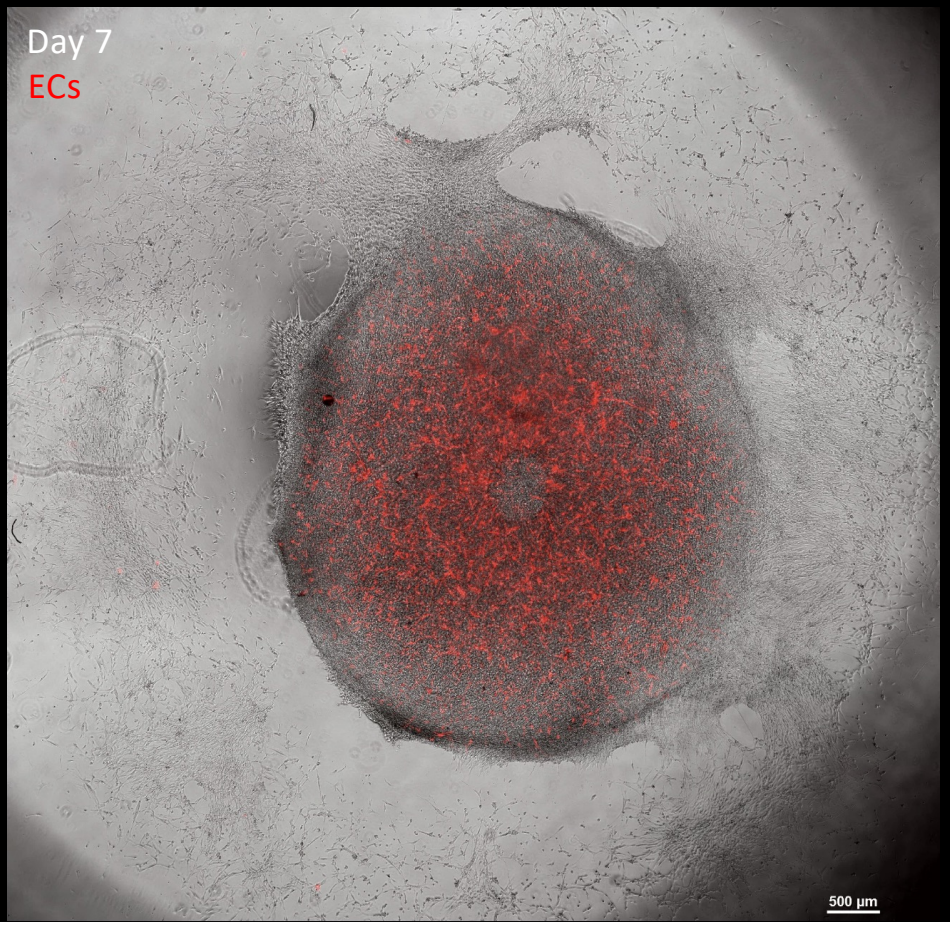
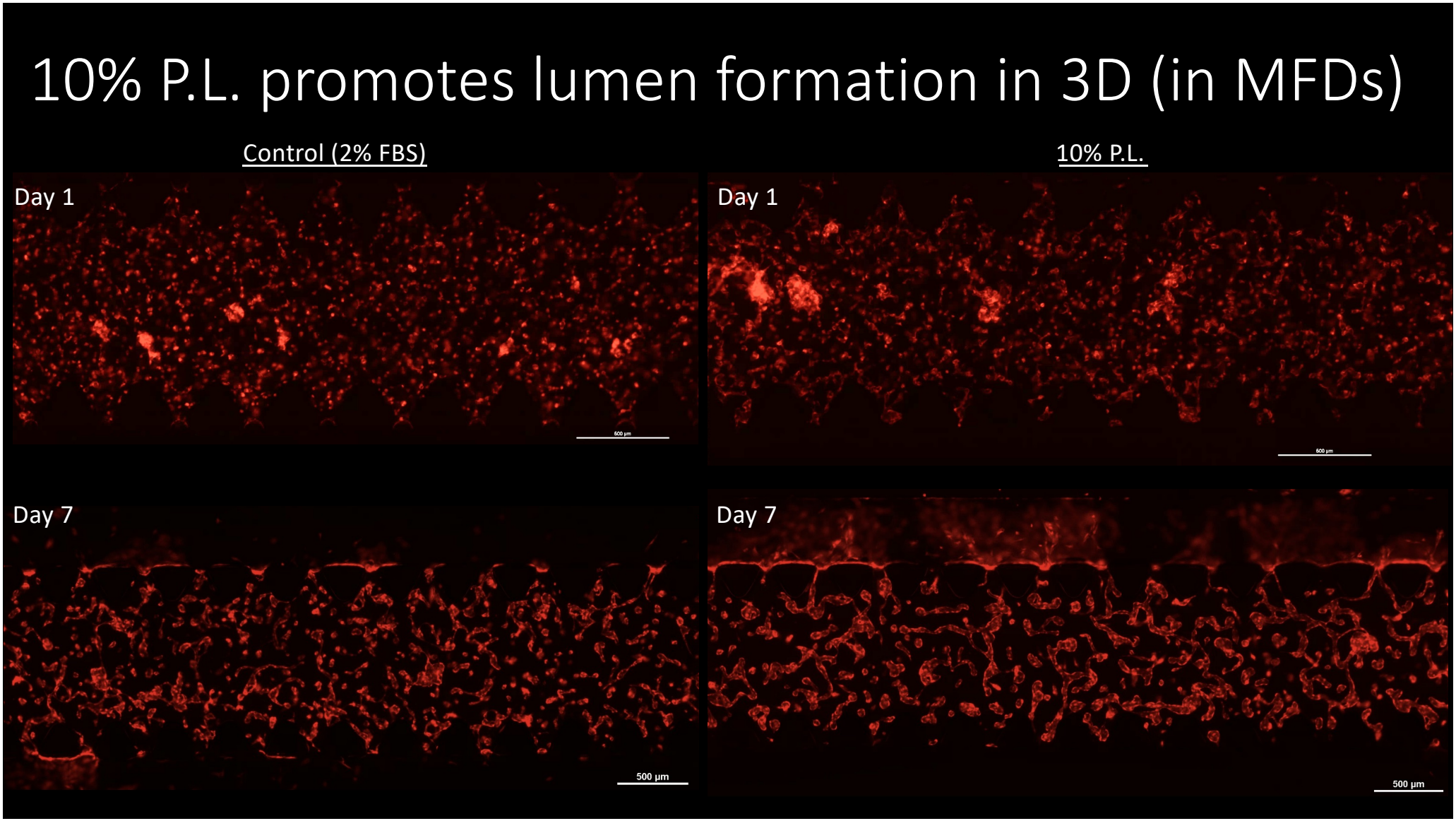


10% P.L.



10% P.L. promotes lumen formation in 3D (in MFDs)



Conclusions

2D Culture

- 10% Platelet lysate was sufficient to replace 2% FBS in our EC culture
  - This condition and the control grew at comparable rate
  - Proper EC phenotype was maintained
    - Cobblestone morphology, minimal signs of EC stress
- 2% and 5% Platelet lysate were insufficient to replace 2% FBS in our EC culture
  - Did not reach 50% confluency at Day 7; grew at much slower rates than the 10% P.L. condition and control
  - ECs demonstrated signs of stress: lots of blebbing observed, cell morphology was improper
    - Large cell bodies, lack of tight-packed cells in cobblestone morphology, “stretchy/stringy” appearance

3D Culture

- 10% Platelet lysate was sufficient to replace 2% FBS in our 3D co-culture with Fibroblasts (n=3 per condition)
  - Supported vascular lumen formation
- Improved connectivity of formed vasculature was observed in 10% P.L. Condition
- Increased fibroblast proliferation observed in brightfield images of 10% P.L. Condition (particularly in fibrin dot controls)