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Children's HEART
Research
and Outcomes Center
HERO

THE SERPOOSHAN LAB

COMPARATIVE STUDY OF PLENTY SERUM VS. REGULAR FBS IN 2D AND 3D CELL CULTURES

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Experimental Design

- **2D and 3D cell culture using Plenty serum vs. regular Fetal Bovine Serum (FBS)**
- **Cell Lines:**
 - Human Umbilical Vein Endothelial Cell (HUVEC)
 - HepG2 (a human liver cancer cell line)

Cell Line	HUVEC	HepG2
Culture Duration	5 days	15 days
Culture Media	VascuLife® Growth Medium	<ul style="list-style-type: none">• Dulbecco's Modification of Eagle's Medium (DMEM)• 10% regular FBS or Plenty serum• 1% Pen/Strep• 1% MEM Non-Essential Amino Acids Solution
Media Change	Every 3 days	Every 3 days

Experimental Design (con.)

- **2D Cell Culture of HepG2s**

- HepG2 monolayer cells were seeded in 6-well plates in two groups:
 1. Control group using media containing regular FBS ($n = 6$)
 2. Experimental group using media containing Plenty serum ($n = 6$)
- Seeding density: 200k cell/well
- Media: 3 mL per well; changed every 3 days
- Assessment
 1. Optical imaging every day
 2. Cell viability analysis ($n = 3$ / time point) on days 7 and 15

Experimental Design (con.)

- **2D Cell Culture of HUVECs**

- HUVECs were seeded in 6-well plates in two groups:
 1. Control group using media containing regular FBS ($n = 6$)
 2. Experimental group using media containing Plenty serum ($n = 6$)
- Seeding density: 50k cell/well
- Media: 3 mL per well; changed every 3 days
- Assessment
 1. Optical imaging every day
 2. Cell viability analysis ($n = 3$ / time point) on days 3 and 5

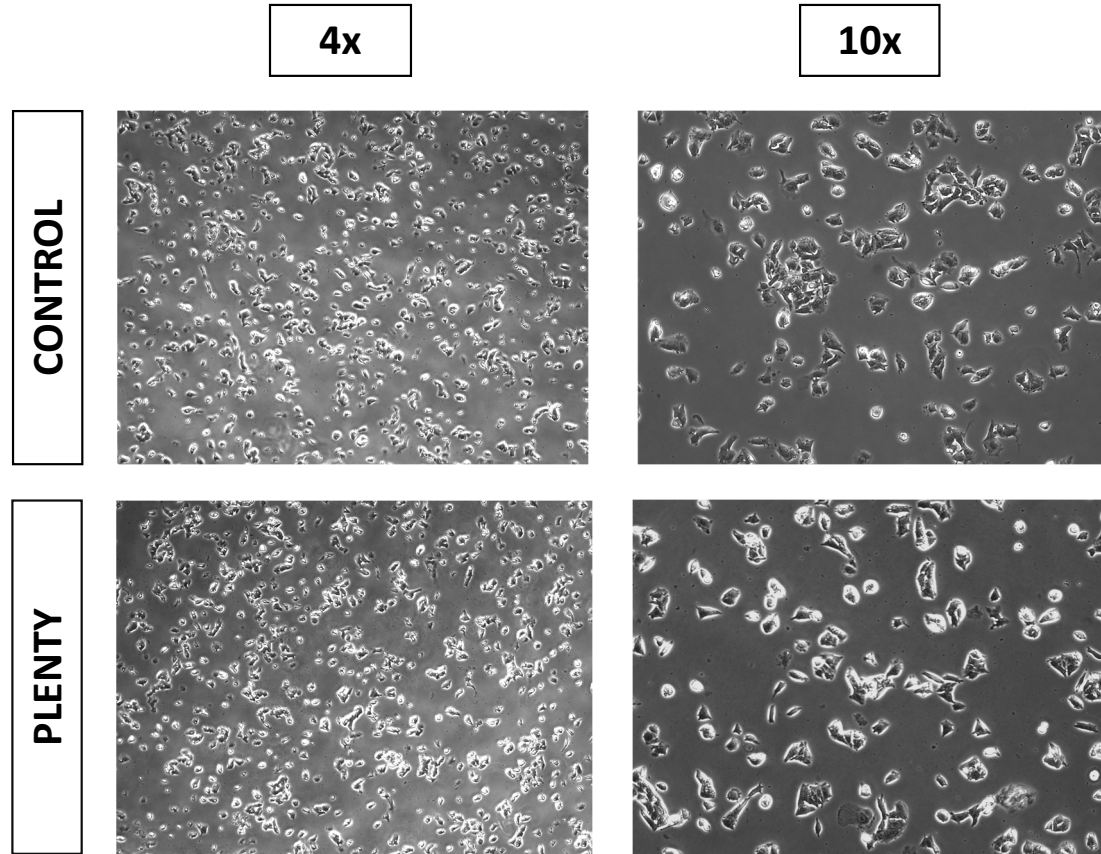
Experimental Design (con.)

• 3D Cell Culture of HUVECs

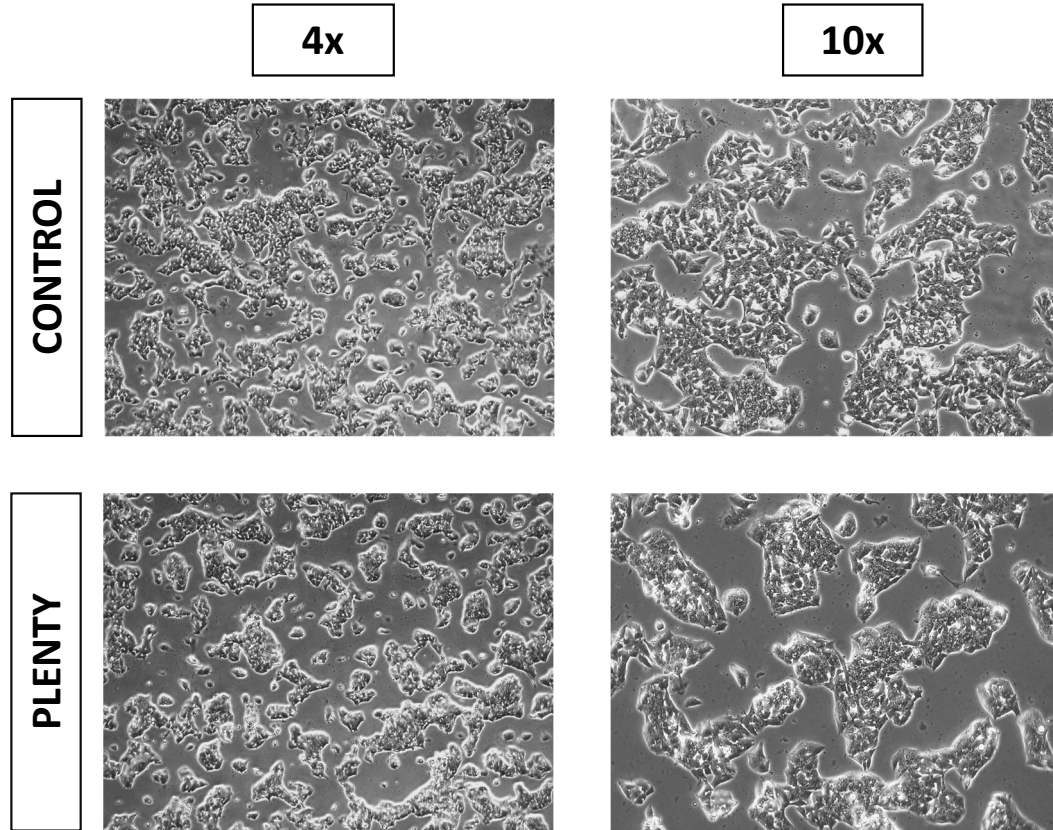
- Gelatin methacrylate (GelMA) mixed with HUVECs was cast in 48-well plate in two groups:
 1. Control group using media containing regular FBS ($n = 6$)
 2. Experimental group using media containing Plenty serum ($n = 6$)
- Seeding density: 200k cell/well
- Final concentrations: 5% GelMA containing 0.5% Irgacure (photoinitiator)
- Crosslinked by UV light at the intensity of 10 mW/cm²; 2 mins
- Media: 500 μ L per well; changed every 3 days
- Assessment
 1. Fluorescent imaging every day
 2. Live/Dead cell viability assay ($n = 3$ / time point) on days 3 and 5

2D Culture of HepG2s

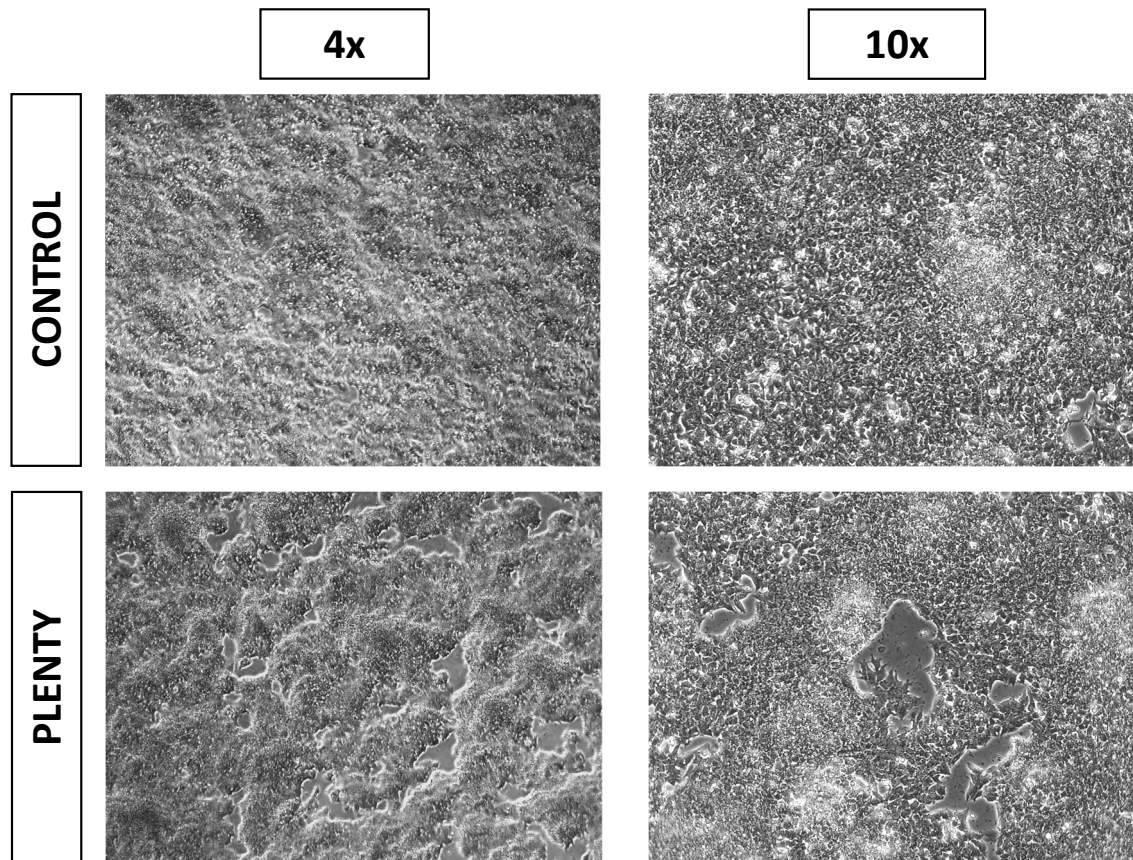
Optical Images of HepG2 2D Culture - Day 1



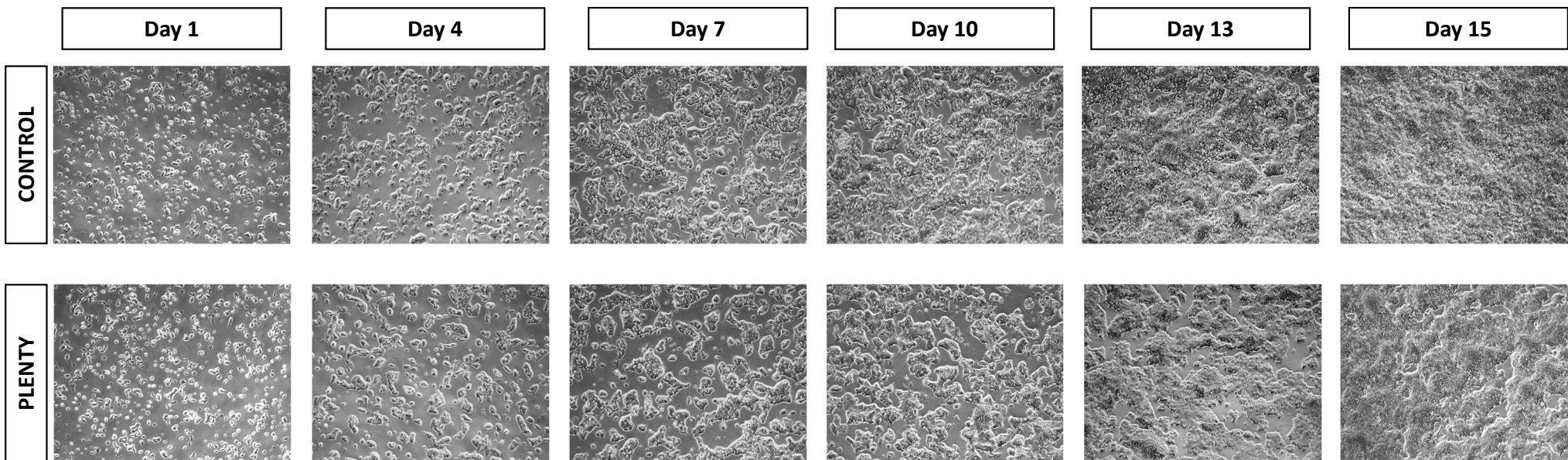
Optical Images of HepG2 2D Culture - Day 7



Optical Images of HepG2 2D Culture - Day 15



Compiled Optical Images of HepG2s (4x)



HepG2 Cell Viability Analysis on Day 7

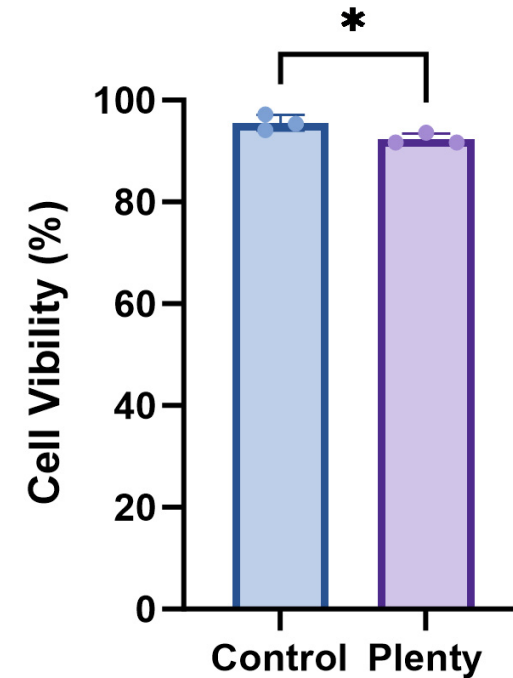
CONTROL

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	1,800,000	113,600	94.1%
2	2,348,000	113,600	95.4%
3	2,485,000	56,800	97.2%
Average	2,211,000	94,667	95.6%

PLENTY

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	416,000	37,880	91.7%
2	628,000	51,600	91.7%
3	832,000	49,600	93.6%
Average	625,334	46,360	92.3%

Cell Viability at Day 7 (HepG2)



HepG2 Cell Viability Analysis on Day 15

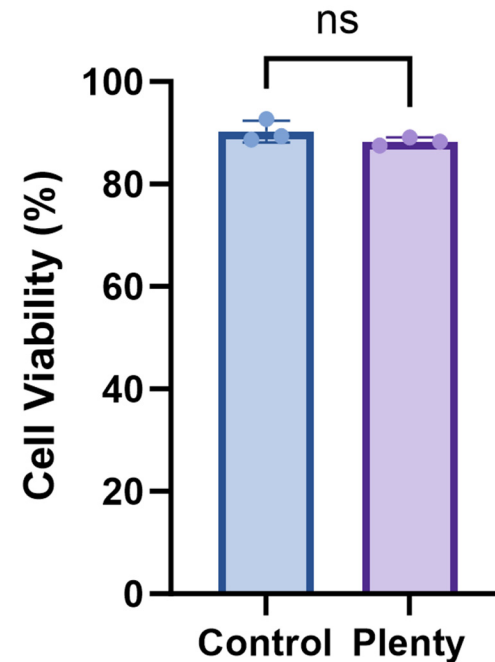
CONTROL

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	8,160,000	964,000	89.4%
2	8,880,000	700,000	92.7%
3	8,000,000	1,019,000	88.7%
Average	8,346,667	894,334	90.3%

PLENTY

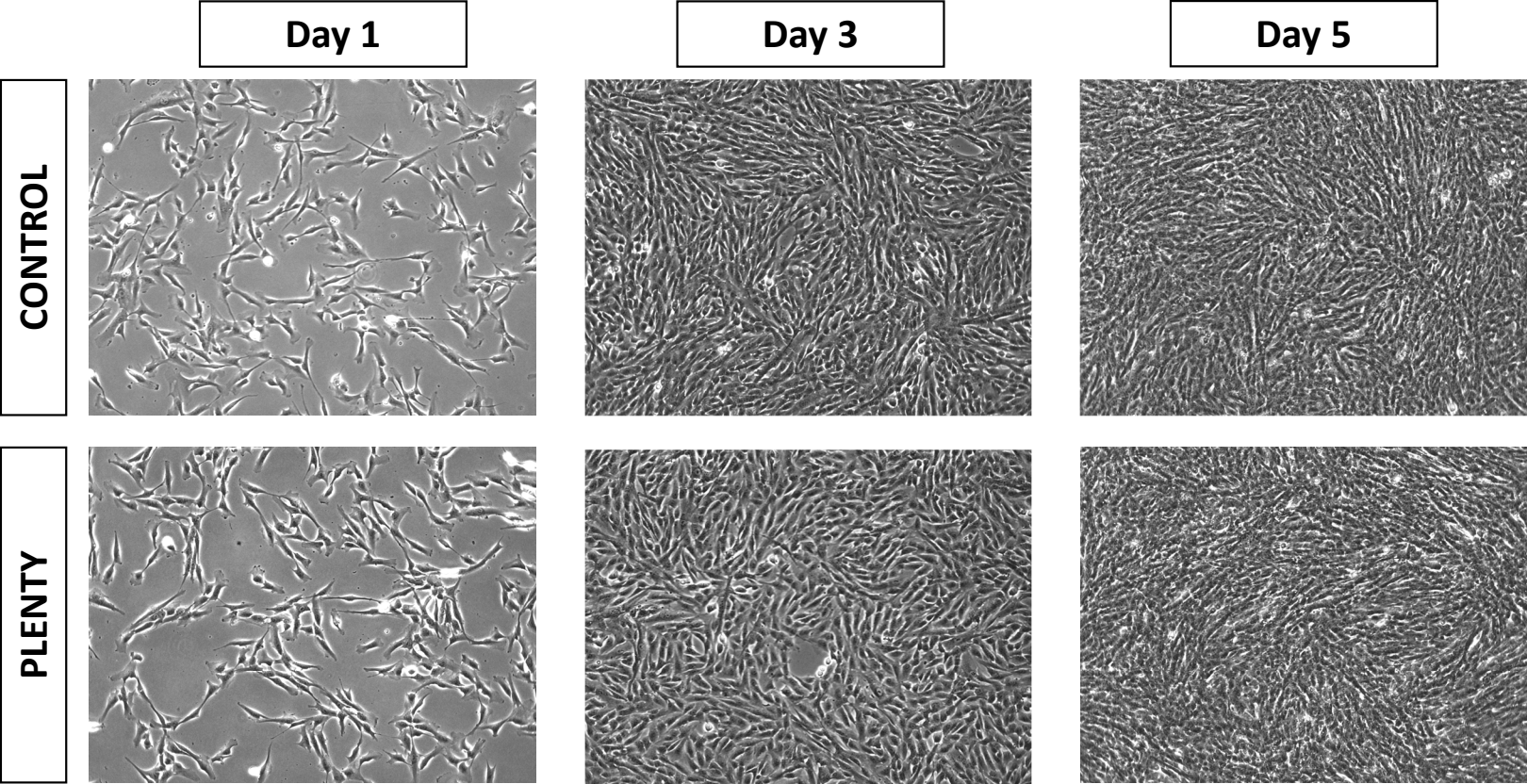
Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	6,400,000	776,000	89.1%
2	7,840,000	1,044,000	88.3%
3	7,280,000	908,000	87.5%
Average	7,173,333	909,334	88.3%

Cell Viability at Day 15 (HepG2)



2D and 3D Culture of HUVECs

Optical Images of HUVECs 2D Culture (4x)



HUVEC Viability Analysis for 2D Cultures on Day 3

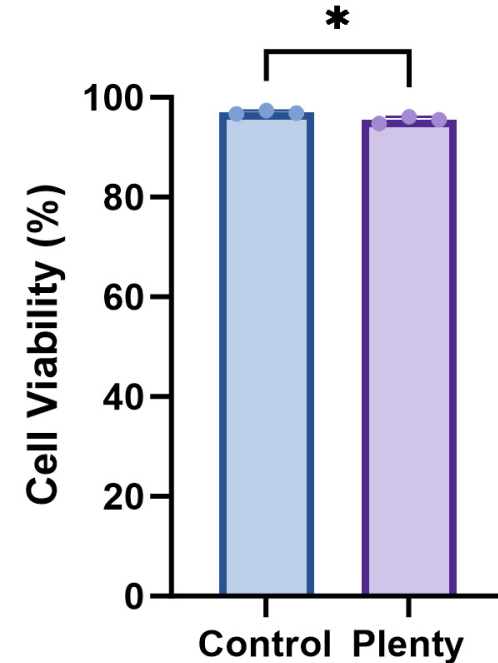
CONTROL

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	1,460,000	37,960	97.4%
2	1,340,000	44,200	96.7%
3	1,620,000	50,200	96.9%
Average	1,473,333	44,120	97.0%

PLENTY

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	960,000	37,420	96.1%
2	1,100,000	49,510	95.5%
3	1,030,000	53,560	94.8%
Average	1,030,000	46,830	95.5%

Cell Viability at Day 3 (HUVECs)



HUVEC Viability Analysis for 2D Cultures on Day 5

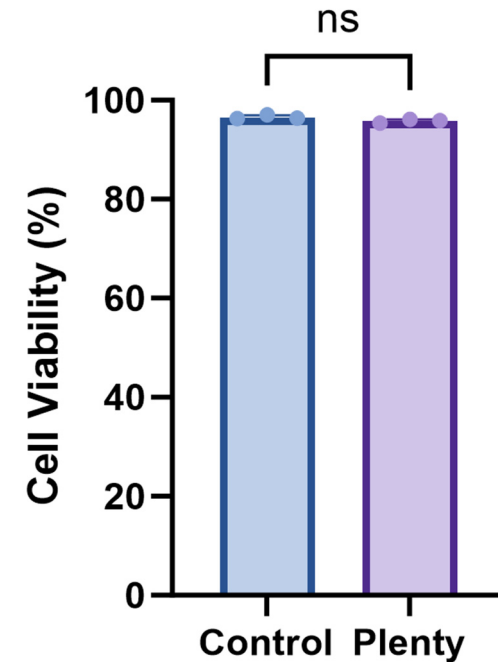
CONTROL

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	1,720,000	66,291	96.3%
2	2,190,000	81,784	96.4%
3	2,070,000	64,020	97.0%
Average	1,993,333	70,698	96.6%

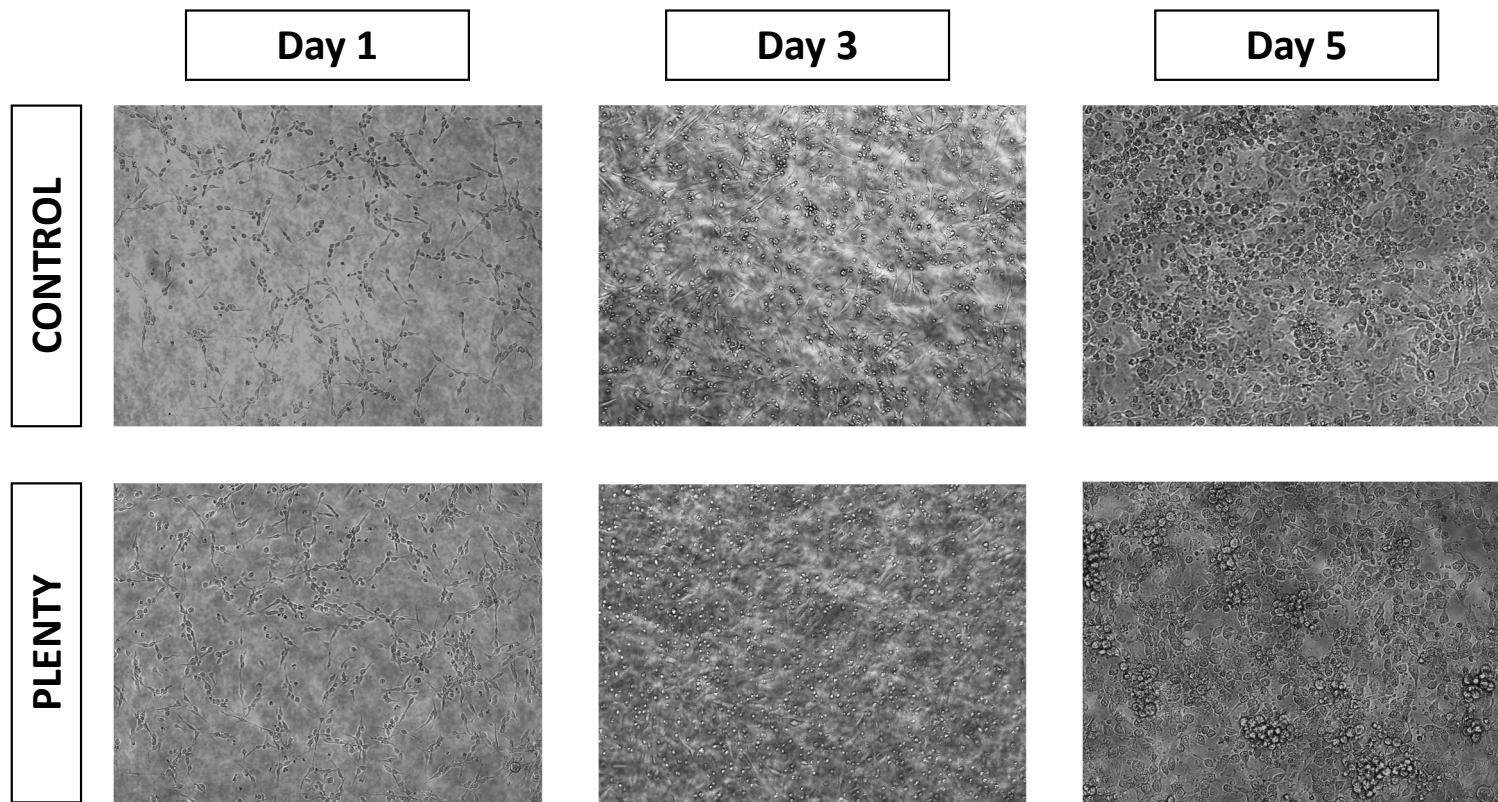
PLENTY

Sample	Live Cells (cells/well)	Dead Cells (cells/well)	Cell Viability (%)
1	1,600,000	77,148	95.4%
2	1,340,000	54,381	96.1%
3	1,690,000	72,252	95.9%
Average	1,543,333	67,927	95.8%

Cell Viability at Day 5 (HUVECs)

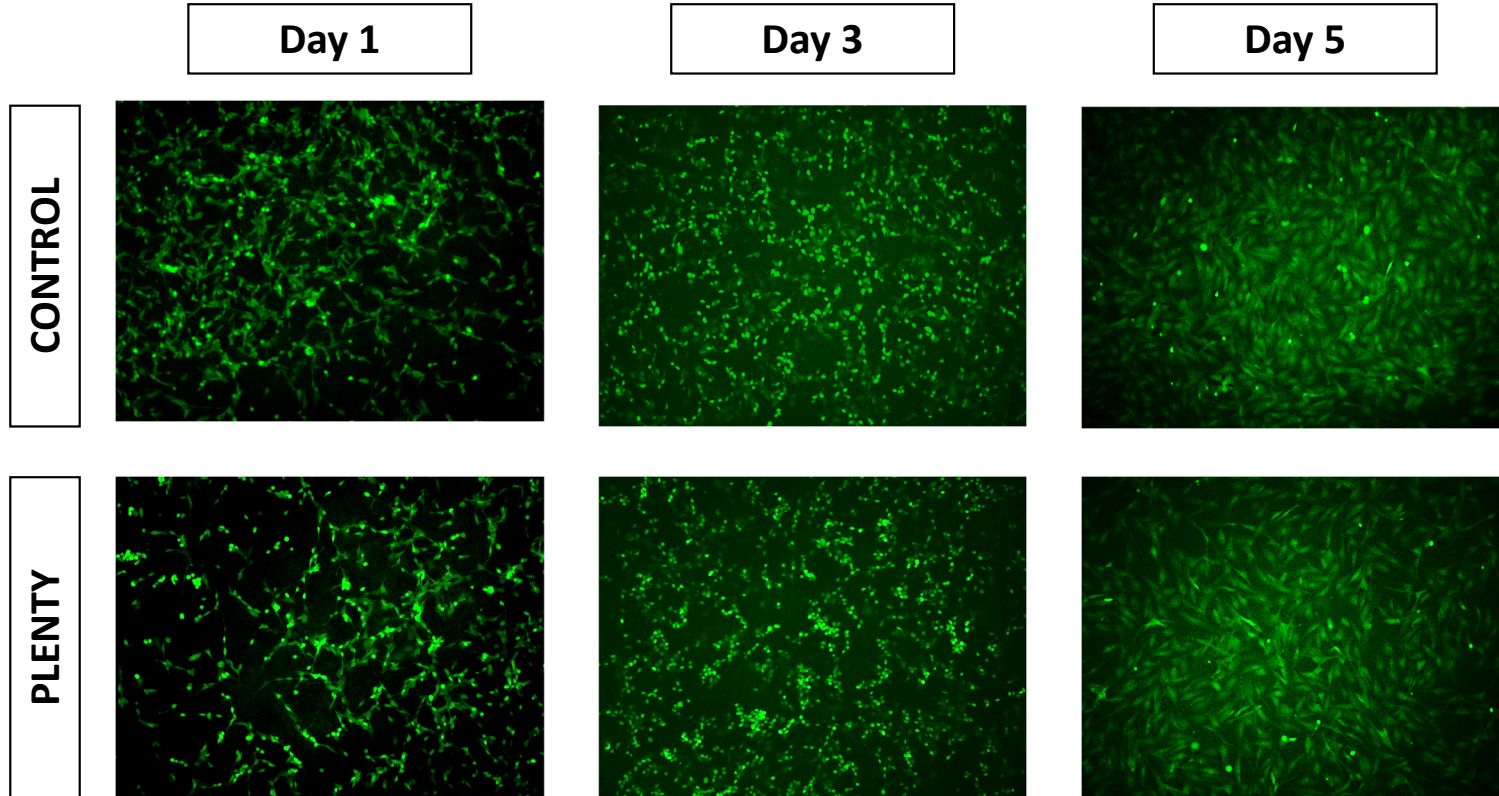


Optical Images of HUVECs 3D Culture (10x)

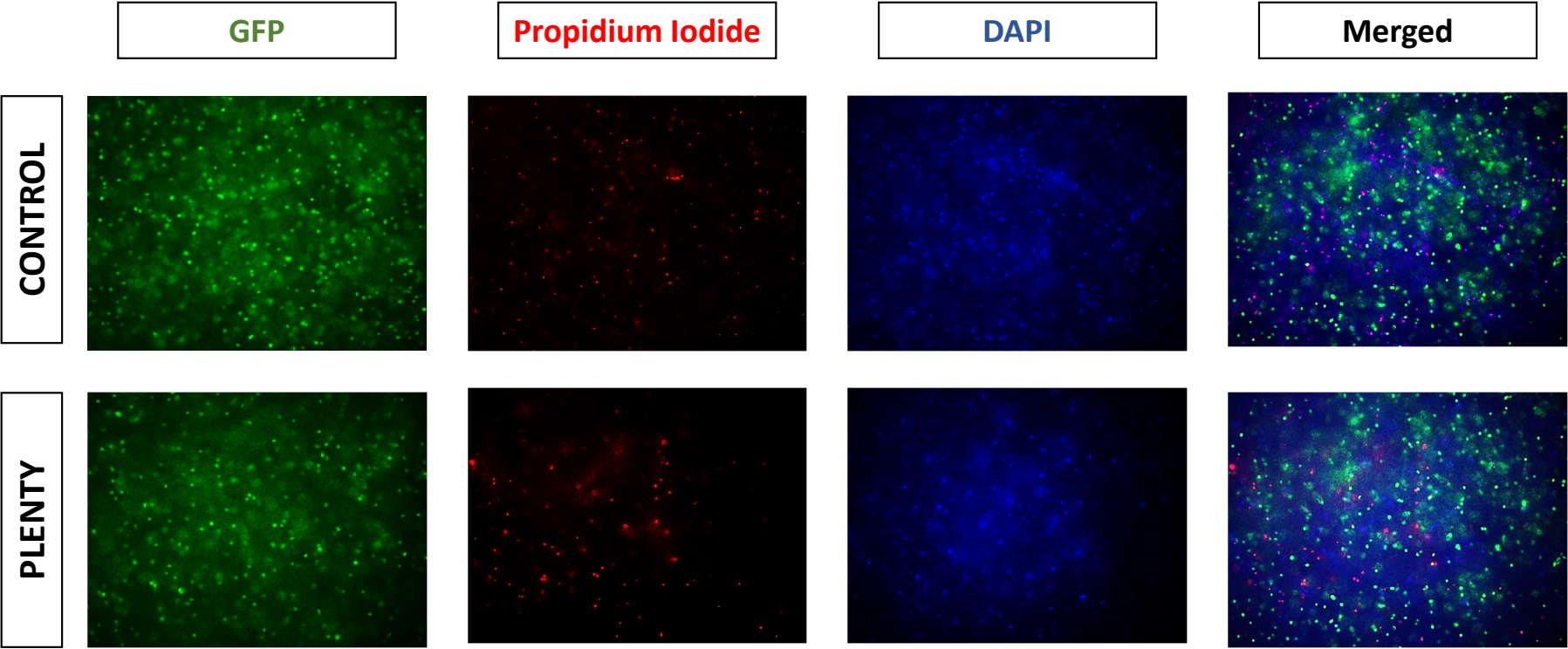


Fluorescent Images of HUVECs 3D Culture (10x)

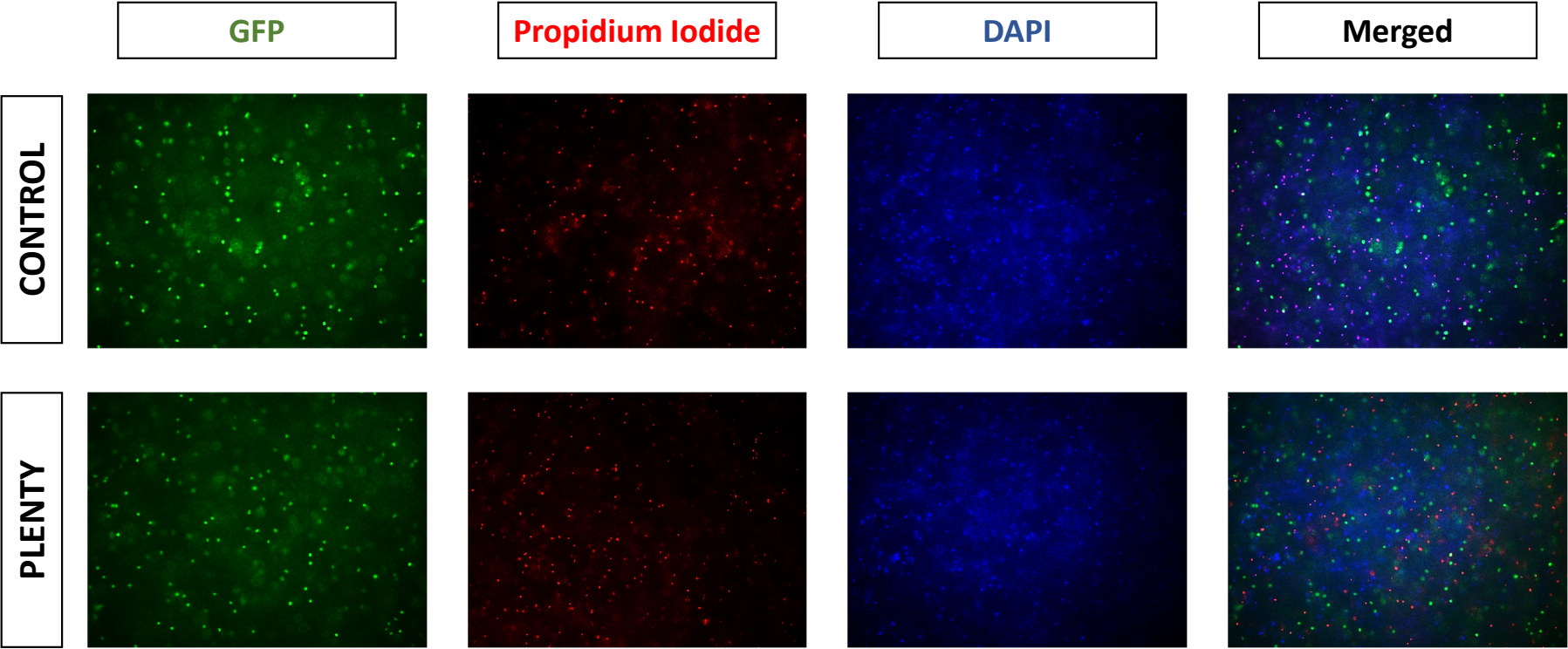
Using pre-labeled GFP+ HUVECs



Live/Dead Assay Images of HUVECs 3D Culture (10x)- Day 3



Live/Dead Assay Images of HUVECs 3D Culture (10x)- Day 5



Conclusions

- No significant differences were observed in the time needed to reach confluency in 2D culture for both cell lines (HUVECs and HepG2 cells) when cultured with regular FBS vs. Plenty serum.
- No significant differences were observed in the viability of HUVECs and HepG2 cells in both control and Plenty serum groups in the 2D cultures.
- The 2D growth rate of cells in the control FBS groups was slightly, but significantly, higher than that in Plenty groups at earlier culture times. At later time points, there were no differences in the cells' proliferation rate.
- When cultured in 3D hydrogels, both cell lines showed no noticeable (qualitative) differences in their viability and growth between the control FBR vs. Plenty serum groups.