Characterization and Tracking of Immune Cell Dynamics as a Model Study for a Placenta-on-a-chip Platform

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Introduction

- The placenta plays a crucial role in pregnancy success by nourishing the fetus, preventing diseases, and avoiding fetal rejection.
- Key aspects of placental immunology and nutrient transport remain poorly understood, limited by ethical constraints on human research and inadequate animal models that fail to mimic human placental structures.
- In vitro models lack the 3D and multi-layered complexity of the placenta, and ex vivo models offer limited insights into early-gestation stages.
- Current placenta-on-a-chip models offer a controlled, ethical research platform but lack precision in representing early gestational stages.
- To address this, we are developing a 3D-printed microfluidic chip that models early-stage placental interactions between placental and immune cells.
- Before creating the full chip, we are characterizing 3D-cultured JAR cells (placental cells), HEK cells (placental transporting epithelium), and THP-1 monocyte cells (innate immune cells) in a degradable PEG-RGD-VPM hydrogel, which closely mimics placental architecture.
- The 3D culturing approach allows us to study cell behavior in a more physiologically relevant context, enhancing our understanding of early placental conditions.
- THP-1 cells are used to assess migration into JAR-seeded hydrogels.
- THP-1s differentiated to M0 macrophage cells to analyze cytokine secretion and interactions with JAR cells to model immune-trophoblast crosstalk.
- This study aims to advance early placenta knowledge and contribute to future drug development and treatment options for pregnancy complications; ultimately improving fetal and maternal health.

Methods

- JAR cells were cultured in RPMI 1640 media with supplements to model placental trophoblasts, while HEK cells, representing the control placental transporting epithelium, were cultured in Advanced DMEM media with similar supplements. THP-1 monocytes were cultured in RPMI 1640 with the same supplements in addition to β-mercaptoethanol (1000x dilution). All cells were maintained at 37°C in a 5% CO_2 incubator and passaged at 60-90% confluence.
- A separate group of THP-1 cells were differentiated into M0 macrophages through a 24 hour incubation in PMA for co-culture with JAR cells to examine cytokine TNF-α secreted by cells.
- PEG-MAL, RGD, and VPM reagents were used for degradable synthetic hydrogels throughout experimentation.
- 80,000 JAR and HEK cells were seeded individually to a non-treated 48-well plate in hydrogels for Alamar Blue assay.
- The Alamar Blue assay was performed to assess cell viability and metabolic activity within the hydrogels via fluorescence reading. This process was repeated on days 2 and 3 to track cell viability over time.
- The JAR cell hydrogels used for the Alamar Blue assay were recreated and stained with 0.1μL of Calcein and 0.5μL of Ethidium Homodimer to track living and dead cells respectively after 3 days of incubation and imaged using confocal microscopy.
- Prior to cell migration and interaction studies, new JAR cells were stained with eFluor 670, and THP-1 cells with eFluor 450. 70,000 JAR cells were seeded in 9 individual hydrogels across 3 non-treated 48-well plates (3 per plate) along with 9 blank control hydrogels. 35,000 THP-1 cells suspended in media were added to each hydrogel-well afterwards.
- Confocal microscopy was performed on days 1, 3 and 5 to observe cell migration and interactions within the hydrogel. Images were analyzed using ImageJ plug-in BioFormat.
- M0 and JAR co-culture was performed to study cytokine secretions in 4 hydrogels in a transwell with 5,000 M0 cells and 70,000 JAR cells.

Acknowledgements

I would like to thank my project mentor Dr. Weaver for this opportunity to work in her lab and gain so many new skills and insights on a very important and novel project. I would also like to thank Chishiba Chilimba for her extended time, effort, and patience in helping me see this project through.

Results

JAR Human Trophoblast Cell Line Survival and Function in Degradable Synthetic Hydrogels

1a.

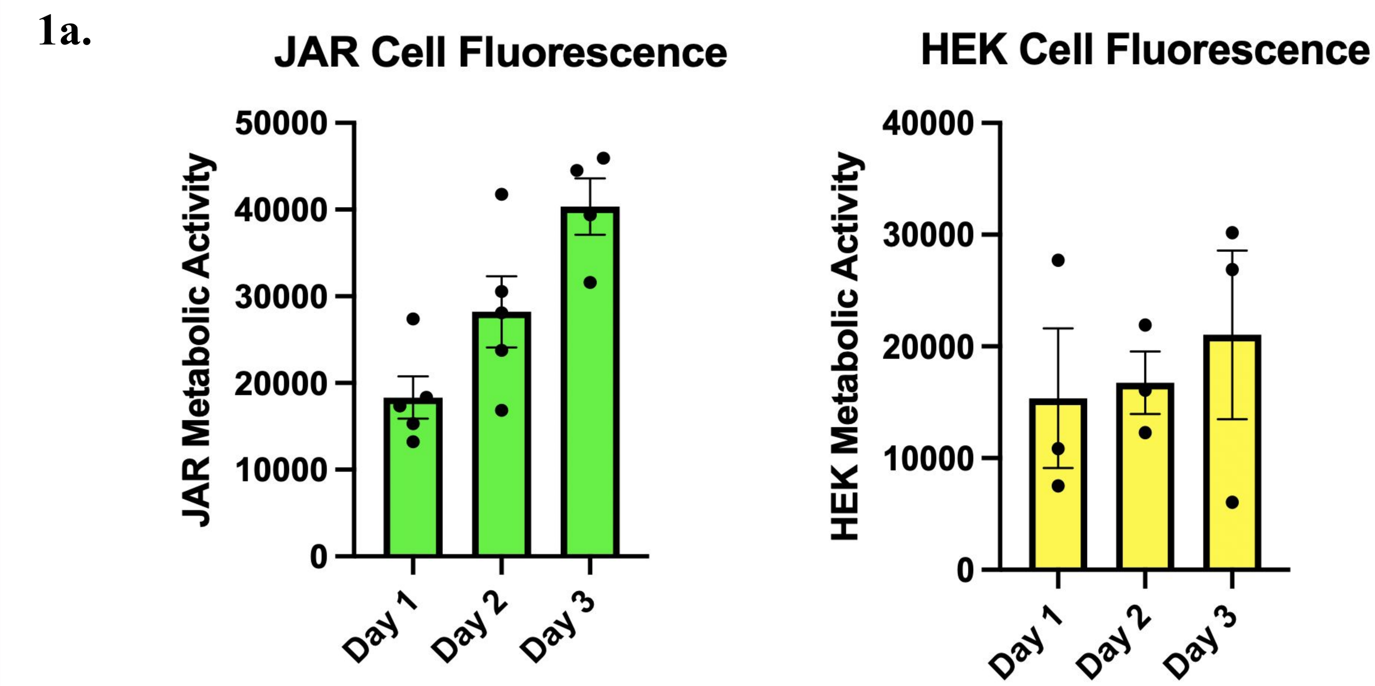


Figure 1a. depicts the average fluorescence of the JAR and HEK cell samples on days 1, 2 and 3 from the Alamar Blue Assay. Fluorescence is indicative of cell viability and metabolic activity, which increased every day.

1b.

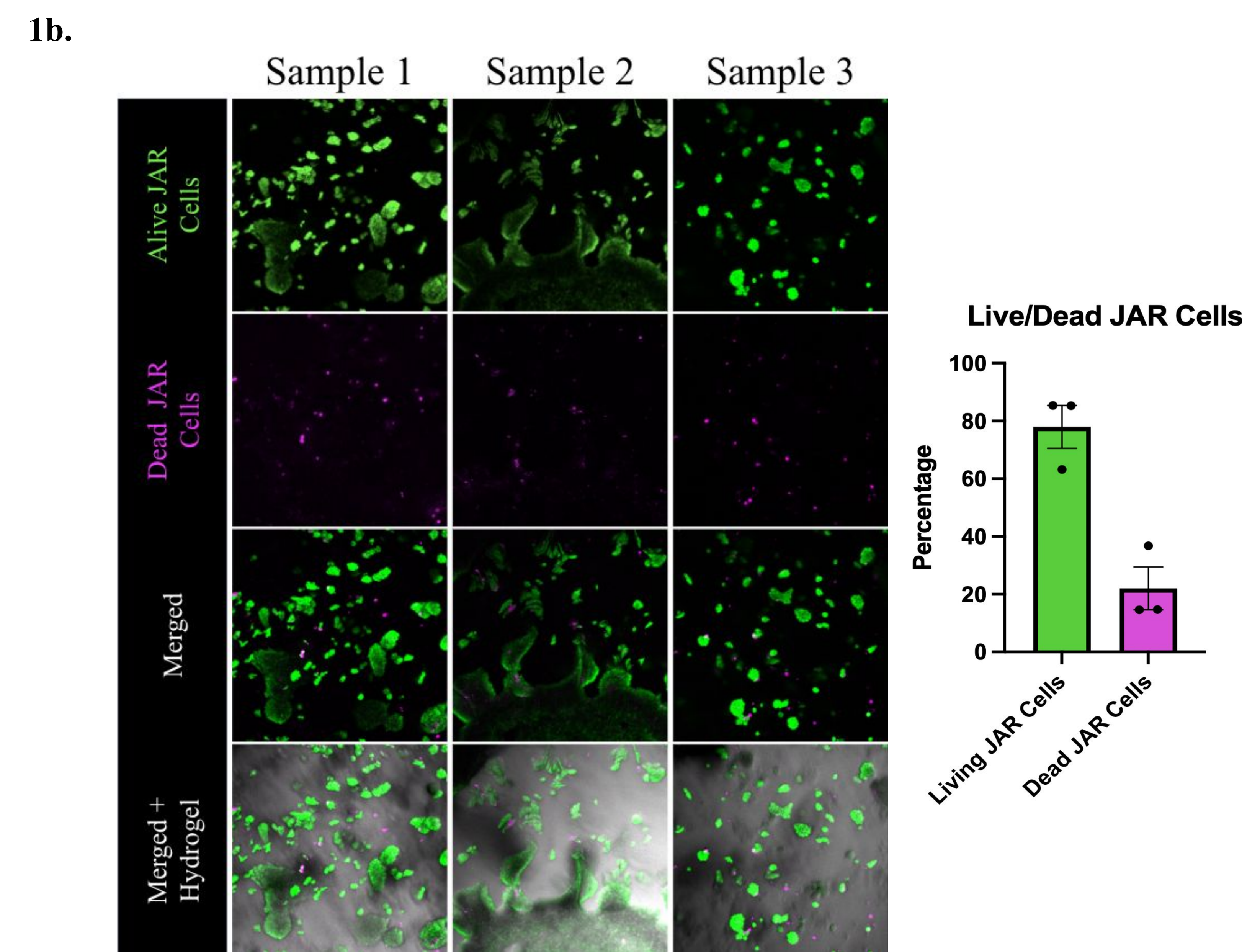


Figure 1b. displays the JAR live/dead confocal microscopy pictures with the live JAR cells in green, and dead JAR cells in magenta after 72 hours within the hydrogel. A merged photo and a bright-field photo are also included for context. Sample 1 had 85.3% living cells, Sample 2 had 85.4% living, and Sample 3 had 63.2% living - averaging to 78.0% living across all 3. These results further demonstrate cell viability within the hydrogels after 3 days of incubation.

Impact of JAR Cell Line on THP-1 Human Monocyte Migration into Degradable Synthetic Hydrogel

2a.

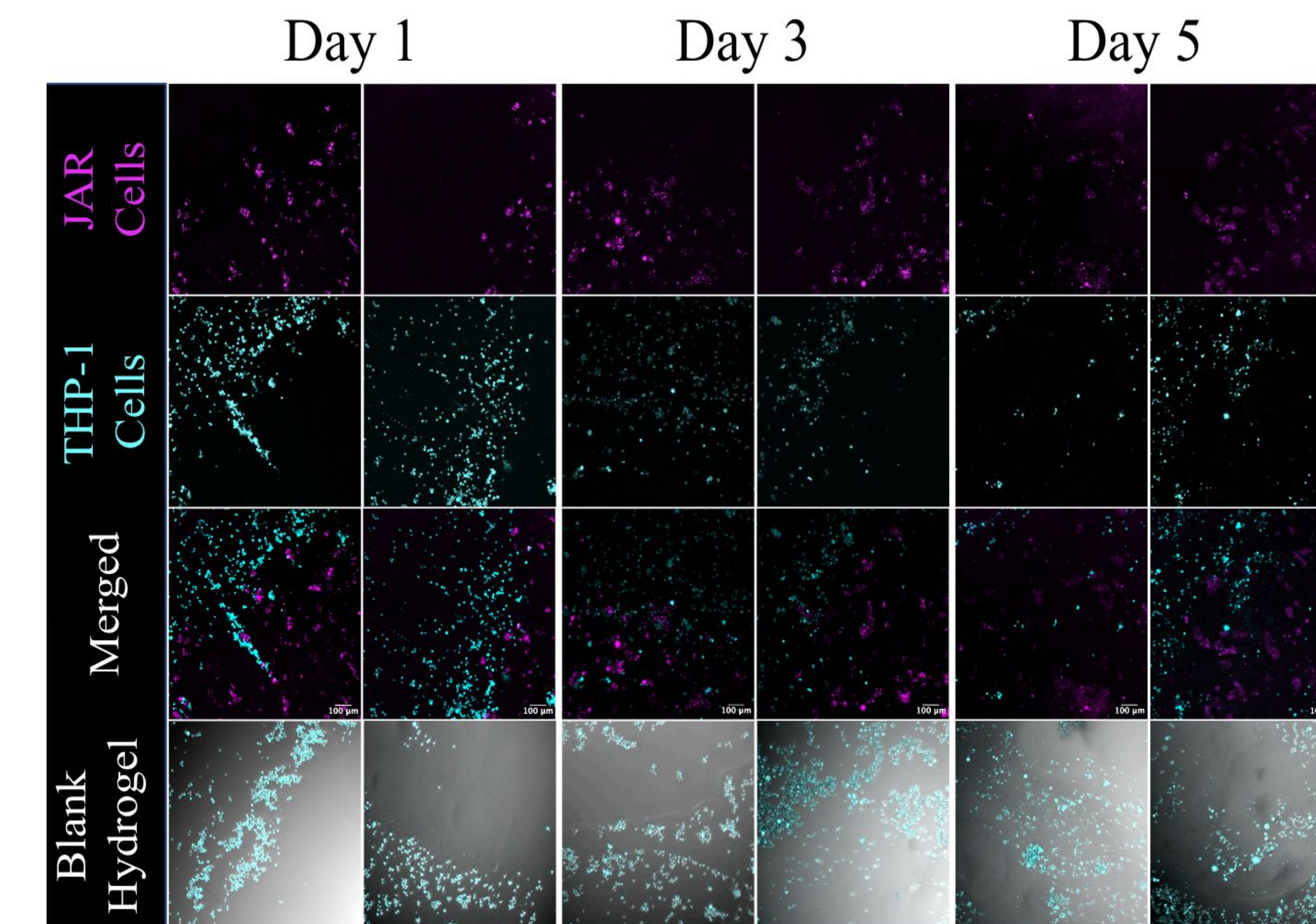
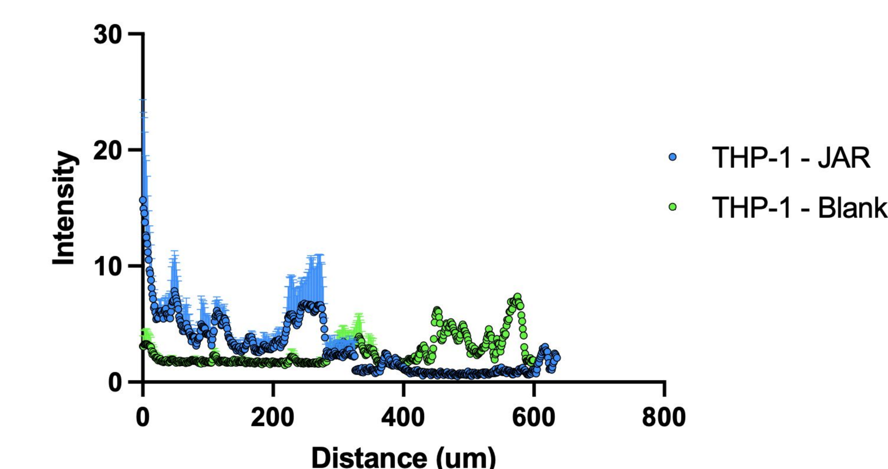
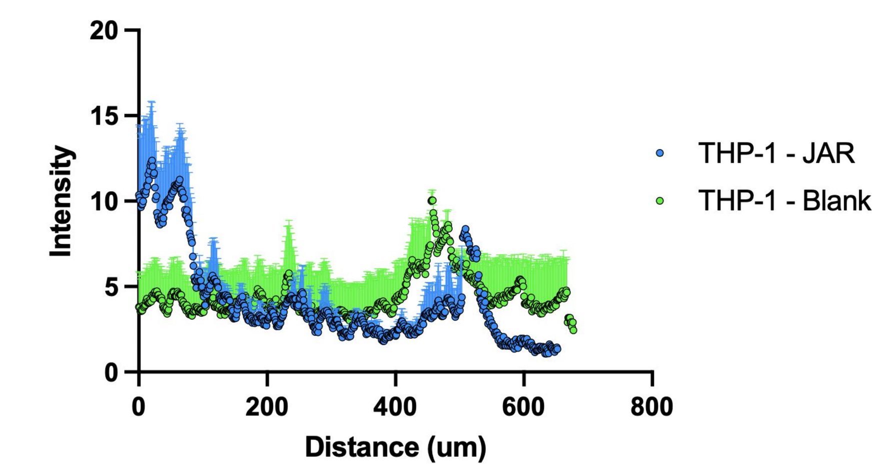
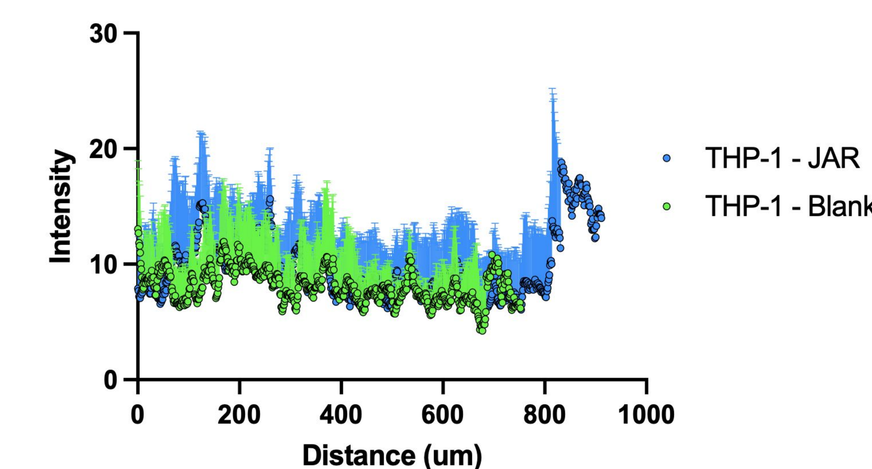


Figure 2a. displays confocal fluorescence microscopy of the JAR-encapsulated hydrogels with added THP-1 cells on days 1, 3 and 5. The JAR cells are magenta colored while the THP-1 cells are cyan colored. The bottom row of pictures includes bright-field images of the hydrogels for context. Pictures were taken as an assortment around the edge of the hydrogel as well as inside of the hydrogel. Data collected from confocal microscopy was used to assess THP-1 cell migration and cell counts into the hydrogel across each day. Scale Bar: 100um

2b.







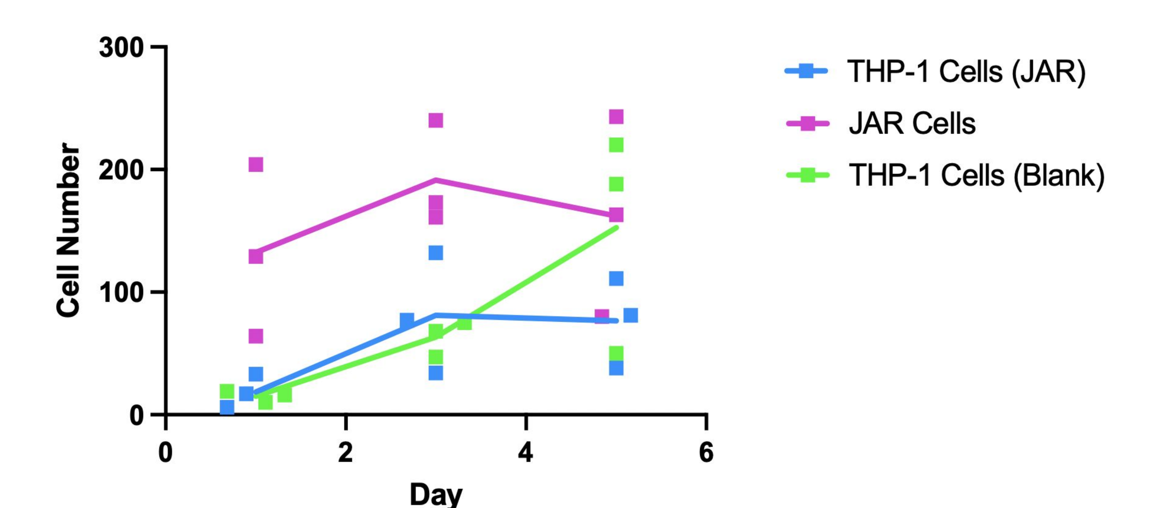


Figure 2b. graphs the average migration distance relative to fluorescence intensity between the THP-1 cells in the JAR hydrogels and blank hydrogels for days 1, 3, and 5. The graphs of the average migration distances between the two experiments suggests that the JAR cells have some modulation in THP-1 behavior, as the THP-1 cells migrated further into the JAR hydrogel relative to the blank hydrogel by day 5. The cell count data further refines this modulation claim as the JAR and THP-1 cells in JAR hydrogels displayed similar patterns in cell counts across the 5 days, while the THP-1 cells in blank hydrogels had significant increase in cell count by day 5.

Effect of JAR Cell Line on M0 Macrophage Pro-inflammatory Cytokine Secretions

3.

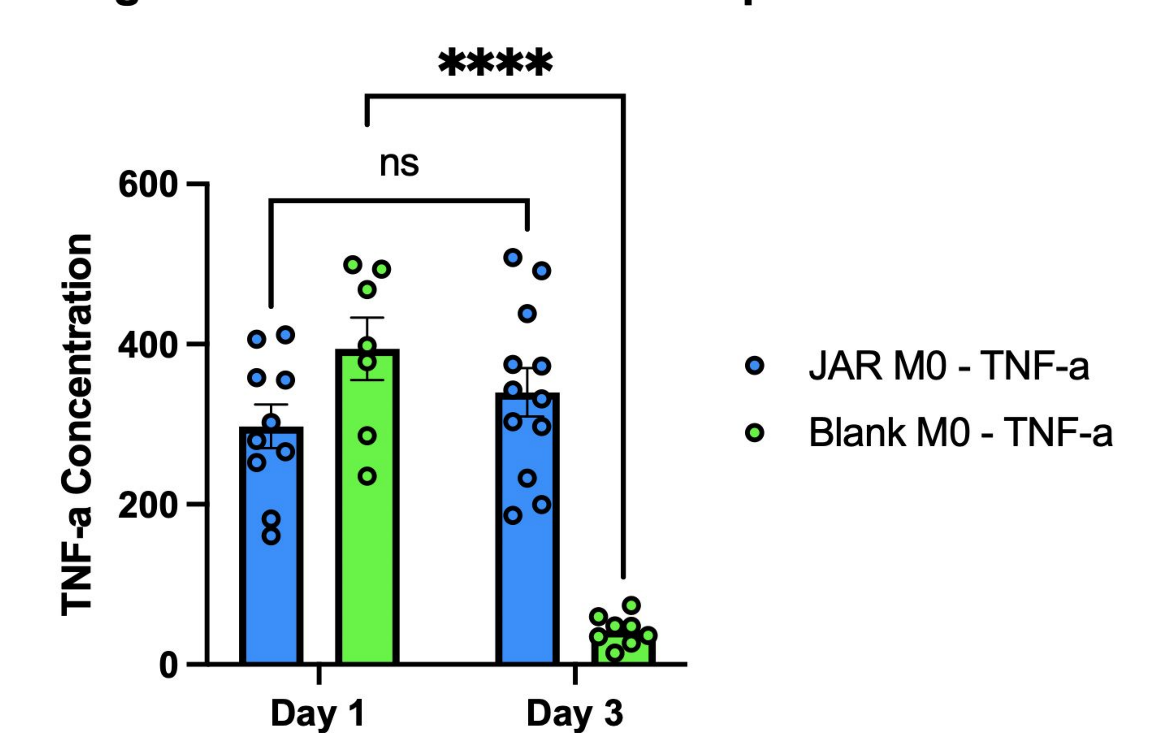


Figure 3. graphs the average secretions of TNF-α across days 1 and 3 for the co-cultured JAR and M0 macrophage hydrogel with a control blank hydrogel via an ELISA. Day 1 secretions in the JAR group displayed lower average concentrations than the blank hydrogel, suggesting some modulation or reduction of M0 TNF-α secretions. Day 3 secretions, however, showed an increased concentration in the JAR group, but drastically lower concentration in the blank hydrogel group. This could indicate JAR cells maintain a more sustained inflammatory environment through interactions with macrophages. TNF-α comparisons analyzed with 2-way ANOVA.

Conclusions

The goal of this project was to examine the cellular communication and immune interactions between placental trophoblast cells and innate immune cells as a model study for a future placenta-on-a-chip platform. We were able to recognize and confirm multiple findings such as cellular metabolic activity within a degradable synthetic hydrogel, THP-1 cell migration and interactions with JAR cells, and TNF-α cytokine secretion comparisons as well. Future work on this project could analyze adaptive immune system cell line interactions with the placental trophoblast cells, as only innate immune cells were examined in this study. Extended studies of cytokine secretions, specifically anti-inflammatory cytokines, could also provide useful insight as a foundational step towards understanding the placental immune dynamics before moving to the placenta-on-a-chip platform.