Enteroid and enterosphere co-culture elicits vasculogeneic response.

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Introduction

Intestinal vasculargenesis in developmental and disease states are not well understood and characterized. It has been found that disease states such as: Inflammatory bowel disease and Celiac disease has a correlation to inflammatory processes that promotes an angiogenic response.[1] Many of these diseases arise from cells residing in the crypt niche, more specifically due to mutations within the intestinal stem cells. Currently, pharmaceutical models lack an *in vitro* culture system to aid in developing treatments for these disease types. This work aims to develop a robust co-culture system which illustrates an elicitation of vasculogenesis from enteroids and enterospheres in which can be parlayed into a pharmaceutical model. The novelty of this system demonstrates the developmental nature of enteroid forming units, derived from intestinal stem cells, and migration of rat aortic smooth muscle cells (RASMC) due to VEGF secretion. Implications of this co-culture system will advance fundamental studies in differentiation as well as propel the tissue engineering field.

Materials and Method

Neonatal Lewis rats were sacrificed through decapitation between ages P2 and P5. Intestinal cell fractions were rinsed five times with PBS and incubated with 25 mL of a 2mM EDTA solution. Intestinal crypts were enriched from the digestate and passed through a 20μm cell strainer for dispersion. Intestinal cell suspension is embedded in Matrigel™ containing 1μM Jagged-1. The matrigel cell suspension was polymerized within a 24-well plate containing confluent layers of RASMCs. The co-culture was supplemented using Sato's procedure[2] with Advanced DMEM-F12™, 50 ng/mL EGF, 100 ng/mL Noggin, and 1 μg/mL R-spondin-1, 0.5μg/mL Wnt3. In positive and negative control populations, the culture wells were additionally supplemented with VEGF and Pl4, respectively. Culture medium was refreshed every 2 days and stored for further ELISA processes. Day progression studies were captured via an inverted microscope.

Results and Discussion

The co-culture experiments were conducted under different scenarios to demonstrate that enteroid and enterospheres were, in fact, eliciting a response to the RASMCs. Internal controls were preformed with RASMCs plated with the respective medium containing the proposed Matrigel without the intestinal suspension. Under these conditions, it was found that the RASMCs proliferated without any negative effects indicating that the medium in addition to the Matrigel addition had little effect. Co-culture experiments were carried out in 3 different conditions and were cultured in parallel. Basal medium, medium containing the necessary factors for enteroid growth, illustrated a migratory response from the RASMCs to the organoid units in culture. The migratory response increased from day 1 to day 2 in culture, but no further migration was seen past day 2. The positive control, Basal medium containing VEGF, illustrated similar migratory patterns in compared the experimental culture (Fig 1 Basal, VEGF). With respect to the negative Pl4 control, an

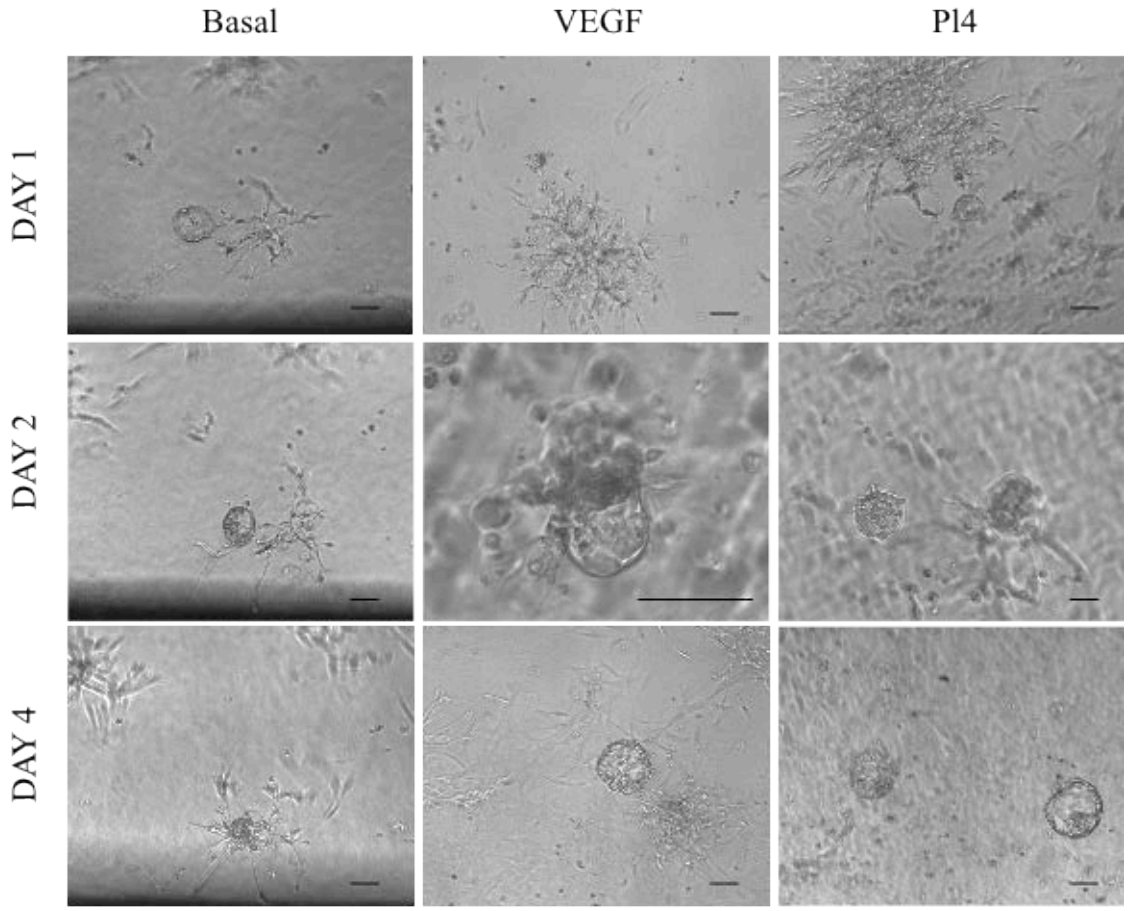

Figure 1 Day progression of enteroid and enterosphere co-culture with RASMCs over a 4 day period. Culture conditions implemented a positive control (VEGF) and negative control (Pl4) each containing the basal medium constituents. Scale bar, 50μm

inhibitor of angiogenesis, little migratory response was found indicating that the experimental (Basal) organoids are in fact secreting an angiogenic cytocine, VEGF. Quantitatively determining VEGF secretion was demonstrated using a conventional ELISA. The VEGF concentration in the Basal co-culture system illustrated a significant increase at day 2 in culture thus coinciding with the images observed (Fig. 1)

Conclusion

Co-culture of enteroids and enterospheres coupled with RASMCs illustrated a significant vasculargenic response in culture and was confirmed quantitatively via ELISA demonstrating that this phenomena is contstrained at day 2 in culture. The co-culture system presented can enable further advances in drug discovery models for intestinal disease states. Furthermore, these studies aid in better understanding of intestinal vascularzation thus promoting advances in the field of tissue engineering and *in vivo* transplantation.

References

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