Stemulate™ supports differentiation of IPS cell-derived Endothelial Colony-Forming Cells

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BACKGROUND

Endothelial colony forming cells (ECFC) are rare circulating and resident vascular endothelial cells that have been isolated from numerous mammalian species (dog, cow, pig, rhesus monkey, baboon, human) that display clonogenic proliferative potential and *in vivo* vessel forming ability. Recently, ECFC have been differentiated from human induced pluripotent stem cells (hiPSC) and human embryonic stem cells (hESC). The hiPSC- and hESC-derived ECFC displayed clonogenic profiles and in vivo vasculogenic capacity similar to umbilical cord blood-derived ECFC. These pluripotent stem cell (PSC)-derived ECFC rescued blood flow to experimentally induced ischemic murine hindlimbs and persisted following injection into tissues as integrated human blood vessels connected to the murine systemic circulation, rescued alveolar lung structure and pulmonary vascular blood flow in hyperoxia exposed newborn mice following intravenous delivery, and integrated into the retinal vasculature in sites of hypoxia to prevent neovascular tufts upon intravitreal injection into hyperoxia challenged newborn mice. Therefore, developing manufacturing protocols for differentiation and expansion of ECFC from PSC precursors are needed in preparation for future human clinical trials.

Stemulate™ is a cell culture media supplement that can support growth in many types of cells and is used as an additive replacement to fetal bovine serum used in many laboratories.

PURPOSE

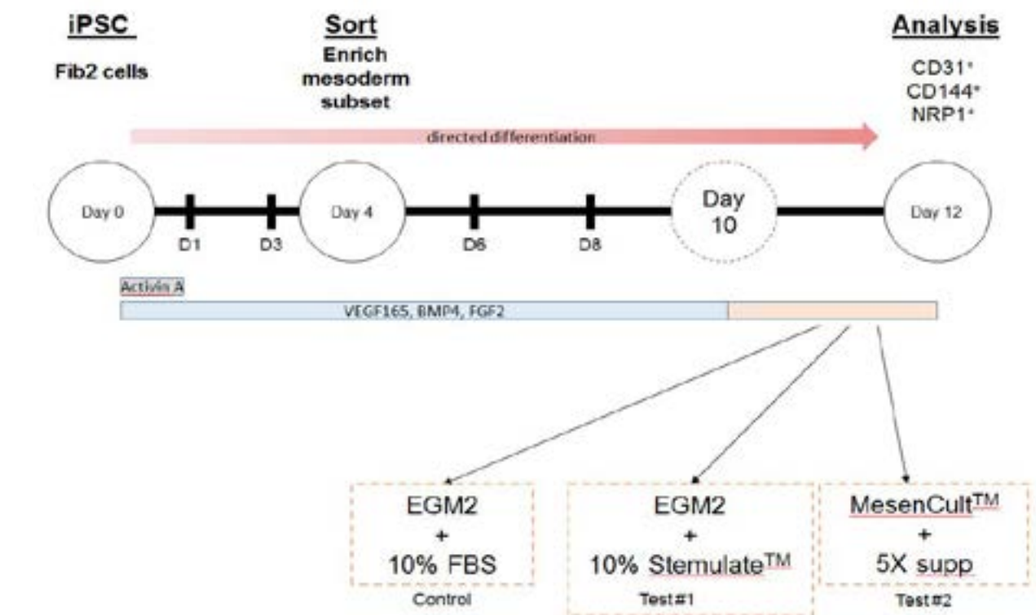
The goal of the present study was to compare the addition of Stemulate™ to Endothelial Growth Media 2 (EGM2) in comparison to addition of fetal bovine serum or MesenCult™ ACF with added 5X supplement with respect to generation of ECFC precursors following in vitro differentiation of hiPSC.

HYPOTHESIS

Stemulate™ will enhance ECFC emergence from differentiated hiPSC in vitro compared to the other culture conditions.

METHODS

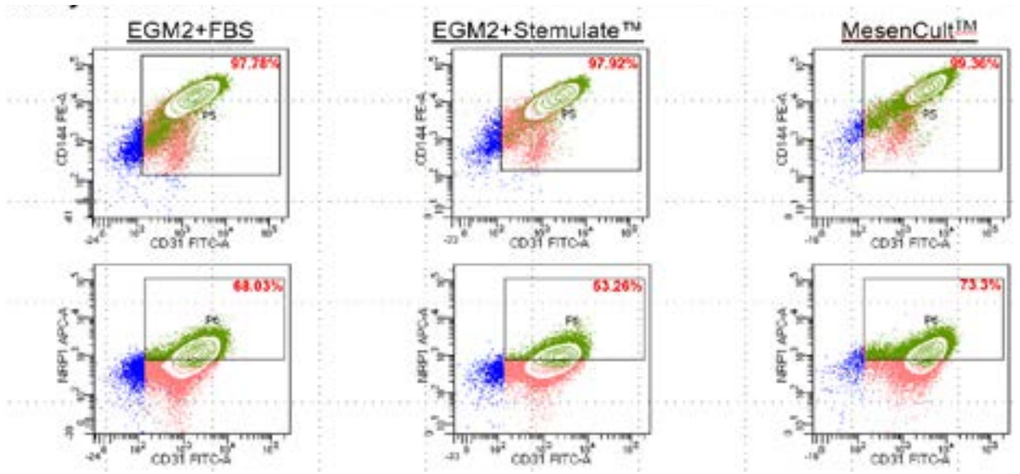
Figure 1. Experimental timeline



- The hiPSC line Fib2 (WiCell®) was maintained in mTeSR1 complete media (Stem Cell Technologies) on Matrigel™ coated 10 cm^2 tissue culture dishes at 37°C and 5% CO_2.
- A 12-day directed differentiation procedure was performed three times comparing three conditions.
- The hiPSC colonies were partially dissociated with dispase and clusters of cells were replated to begin the differentiation procedure.
- The hiPSC were directed toward the mesoderm lineage by activin A (10 ng/mL, first 24 hours only), FGF2 (10 ng/mL), VEGF165 (10 ng/mL) and BMP4 (10 ng/mL).
- A mesodermal subset of cells was enriched by sorting on day 4 of differentiation.
- Stemline® II complete medium (Sigma-Aldrich®) containing FGF-2 (Stemgent®), VEGF165 (R&D Systems®) and BMP4 (R&D Systems) was used to promote endothelial cell emergence and expan-sion.
- Medium was replaced on days 3, 5, 7 and 9.
- Starting on day 10, medium was changed to:
  - EGM2 (Lonza) + 10% FBS, Defined (HyClone™)
  - EGM2 (Lonza) + 10% Stemulate™ (Cook® Regentec)
  - MesenCult™-ACF with 5x supplement (STEMCELL™ Technologies)
- On day 12, cells were dissociated with Trypsin (Gibco®) and immunostained with mouse monoclonal antibodies against CD31 (clone WM59), CD144 (clone 16B1), and neuropilin1 (NRP1)(clone 446921 and AD5-17F6). Samples were analyzed on a Becton Dickinson LSR4 flow cytometer and raw data collected and analyzed using FlowJo® software.
- Data from the three trials were pooled for a sample size of n=3 per condition/study group. Statistical comparisons of means were made pair-wise using T test.

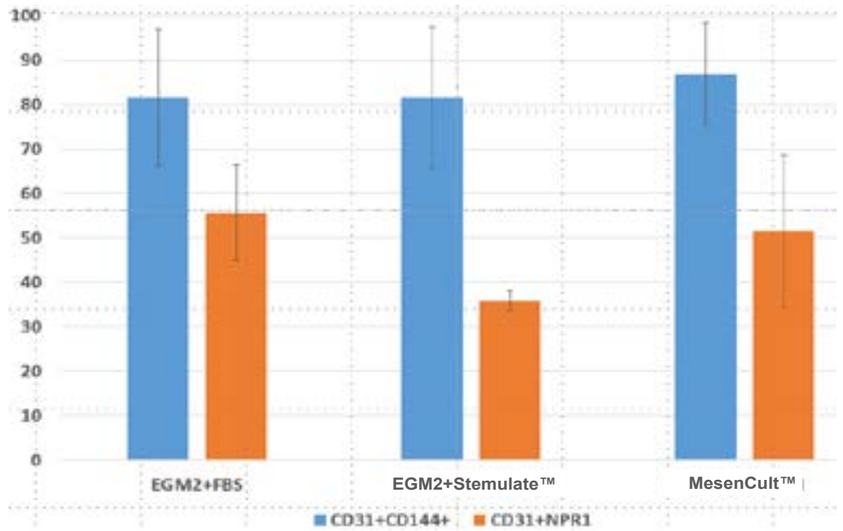
RESULTS

Figure 2. hiPSC-derived ECFC emerge by day 12 in all 3 study groups



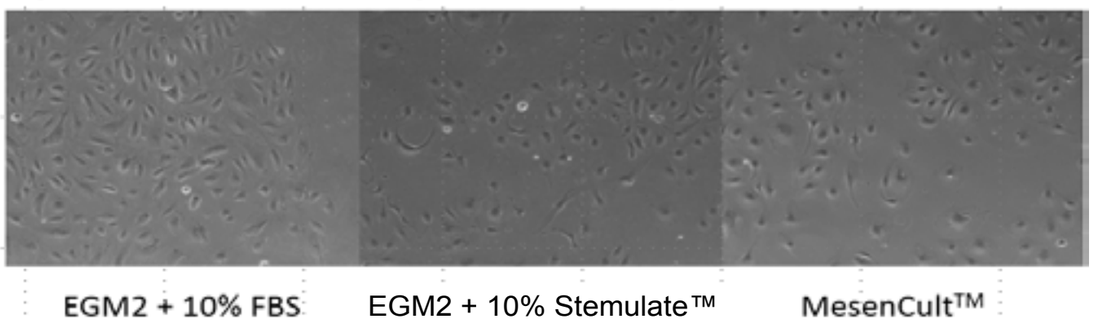
The images depict representative bi-exponential display plots of hiPSC-derived ECFC at day 12 of differentiation for the 3 study groups (EGM2 + FBS, EGM2 + Stemulate™, and MesenCult™). Upper panels display co-expression of CD144 and CD31 and the lower panels display co-expression of NRP1 and CD31. The numbers in red depict the percentage of cells co-expressing the antigens.

Figure 3. Summary of flow cytometric detection of hiPSC-derived ECFC



The bar graphs depict the mean ± SD results of 3 experiments. There is no significant difference in the percentage of cells co-expressing CD31 and CD144 (blue bars) or CD31 and NRP1 (orange bars) among the 3 study groups.

Figure 4. Phase contrast images of cultured day 12 hiPSC-derived ECFC.



Images were obtained using a 10X objective.

CONCLUSION

The data support the use of Stemulate™ as an ethically sourced, xeno-free alternative to FBS for culturing ECFC derived from human PSC using a directed differentiation approach.

CONTACT INFORMATION

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