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REAL TIME MONITORING OF ENDOTHELIAL CELL DYNAMICS TO IMPROVE WOUND HEALING RESEARCH AND EVALUATE THERAPY STRATEGIES

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BACKGROUND

The tube formation assay is a widely utilized in vitro method to evaluate the angiogenic potential of various treatments, especially in the context of wound healing and regenerative medicine. Tube formation assays have been performed using a wide range of endothelial cell types derived from both murine and human sources. When endothelial cells are plated on a reconstituted basement membrane matrix, they rapidly adhere, migrate, and align to form interconnected tubular networks mimicking cell-matrix and cell-cell interactions involved in early vasculogenesis. One of the major limitations in tube formation studies is the lack of standardized parameters, particularly regarding: The number of cells seeded, volume and concentration of Matrigel used ,duration and method of live imaging or endpoint analysis .

OBJECTIVE

In this study, we aim to standardize the tube formation assay by testing a range of HUVEC (Human Umbilical Vein Endothelial Cells) cell numbers (10,000–40,000 cells per well), using a fixed 80 µL of Matrigel matrix enriched with laminin, collagen IV, entactin, and heparan sulfate proteoglycans, and capturing real-time tube formation dynamics over 48 hours through live-cell imaging.

METHODS

HUVECs were cultured in endothelial cell media to 95% confluency. Basement membrane matrix or Matrigel (Corning® Matrigel® Basement Membrane Matrix Growth Factor Reduced) containing various growth factors was pipetted onto a chilled 96-well plate . HUVECs were seeded at densities of 10k, 20k, 30k, and 40k onto the Matrigel-coated plate. The plate was placed in a Sartorius Incucyte imaging system at 37°C and 5% CO_2 for continuous live imaging over 48 hours to monitor endothelial cell migration, alignment, and network formation. Quantitative analysis was performed using AI-based software.

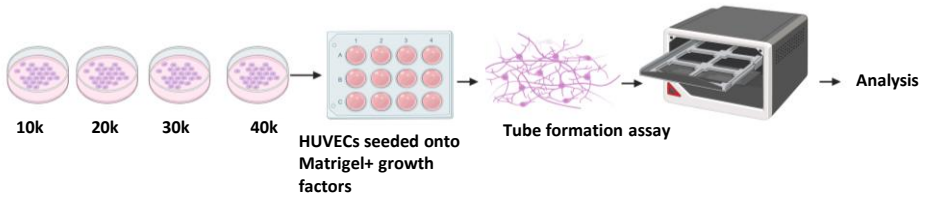

Figure 1: HUVECs were seeded at densities ranging from 10,000 to 40,000 cells per well on a growth factor-supplemented matrix. Tube formation was continuously monitored using the IncuCyte® live-cell imaging system.

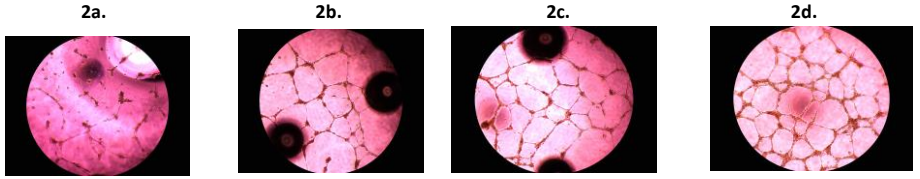

Figure 2: Tube formation assay of Human Umbilical Vein Endothelial Cells (HUVECs) at increasing cell densities (a. 10k, b. 20k, c. 30k, and d. 40k).

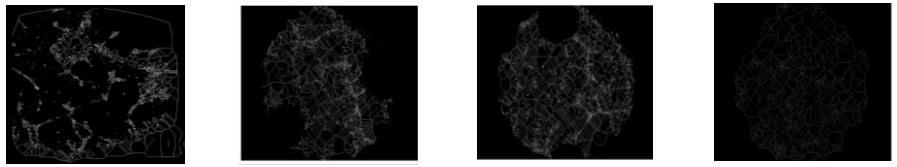

Figure 3: Artificial intelligence-generated skeletal overlays illustrating the structural network formed by cultured Human Umbilical Vein Endothelial Cells (HUVECs) after 24 hours of incubation.

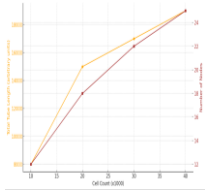

Figure 4: Quantitative analysis of tube formation in HUVECs seeded at increasing densities (10,000 to 40,000 cells per well). Both total tube length and number of nodes peaked in the 40,000-cell group, indicating enhanced network complexity at higher cell densities.

Figure 5: QR code linking to a live video demonstration of dynamic cellular responses. The video captures real-time movement and interaction of human brain microvascular endothelial cells exposed to collagen matrix supplemented with growth factors. Cells display oblique morphology and active migration, highlighting their dynamic behavior under extracellular matrix stimulation.

RESULTS

- ❑ Quantitative analysis showed significant improvements *($p < 0.05$)* in: Total tube length, Number of branching points ,Loop numbers in the 40,000-cell density group compared to lower densities.
- ❑ Angiogenic peak occurred at approximately 24 hours post-seeding.
- ❑ Tube disintegration began between 24 to 36 hours

CONCLUSION

This imaging method, enabling real-time observation of endothelial cell behavior, represents a significant step forward in the study of wound angiogenesis. The system provides high-resolution, continuous live video capture, facilitating detailed analysis of vascular development over time during wound healing. The use of 40,000 HUVECs per well in a 96-well plate, combined with 80 µL of Matrigel (3mg/ml), has proven to be an ideal configuration for ensuring reliable and consistent tube formation in this assay.

LIMITATIONS

The limitation of this study is that we only utilized a single cell line, HUVECs, and did not include other endothelial cell lines, such as murine or alternative human cell lines, which could provide a broader perspective on the angiogenic process.