Corning® PureCoat™ ECM Mimetic Cultureware: Novel Synthetic, Animal-Free Surfaces for Human Endothelial Colony Forming Cell Expansion

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Application Note

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Introduction

Endothelial colony forming cells (ECFCs®) have potential uses in regenerative medicine, such as cell therapy for cardiovascular disease, or use as biomarkers to assess disease risk[1-9]. Expansion of these cells requires coating of a culture surface with human or animal-derived extracellular matrix (ECM) protein[2,8,9], which can introduce pathogens into the culture. Native ECMs can be poorly defined and may have batch-to-batch variability. For all of these reasons, chemically defined extracellular matrix-based systems are required.

To support such research areas, we have developed two synthetic, animal-free (free of human or animal-origin component) peptide surfaces: Corning PureCoat ECM Mimetic Cultureware Collagen I peptide and Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide. Both of these scalable surfaces mimic native ligands for cell adhesion as demonstrated by attachment of specific integrin expressing cell lines. Peptide coated surfaces can be utilized to grow and expand ECFCs, and are stable at room temperature.

Human ECFCs were cultured on these surfaces in ECFC growth medium for multiple passages. Cell attachment, morphology, growth, and viability were comparable to those on freshly coated natural ECM proteins Collagen I or Fibronectin. Cell functionality was demonstrated using a well established angiogenesis tube formation assay[1,5,10], where ECFCs post-expansion formed capillary tube-like structures on Corning Matrigel® basement membrane matrix. ECFCs also demonstrated the ability to ingest acetylated low density lipoprotein (AcLDL), a characteristic feature of endothelial cells[1,2,5,6,9]. Our results suggest that these breakthrough next-generation cell culture environments can be used for the culture of ECFCs in basic and applied research.

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Materials and Methods

Cell Culture and Passaging

Human ECFCs (Poietics™ ECFCs® Endothelial Colony Forming Cells, Lonza) were cultured in ECFC Growth Medium (EBM™-2 plus EGM™-2 SingleQuots™ plus ECFC Serum Supplement, Lonza). Cells were thawed directly onto surfaces at 100,000 cells per well (9.6 cm^2/well) of six-well plates. At passage two and beyond, seeding densities were 50,000 and 100,000 cells per well. Every 2-3 days, cells were passaged according to product guidelines for use or medium was changed. At each passage, cell counts were performed in triplicate using a Countess™ Automated Cell Counter (Life Technologies).

Functional Assays - Cell Staining and Imaging

Cells growing on Collagen mimetic and Fibronectin mimetic surfaces and ECM-coated surfaces were stained with 10-20 µg/mL DiI acetylated low-density lipoprotein (DiI AcLDL, Life Technologies) in ECFC growth medium for four hours at 37°C, and then counterstained with 1.5 µg/mL DAPI (Life Technologies) following manufacturer's instructions.

Images were acquired using an Olympus® IX70 fluorescence microscope with a QICAM™ Mono 10bit digital microscope camera (QImaging™) and processed using QCapture™ Pro software (Media Cybernetics).

Functional Assays - Tube Formation

Cells were plated onto the Corning BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation (Cat. No. 354149) at 20,000 to 80,000 cells per well in growth medium and incubated overnight at 37°C. Images were captured using an Olympus IMT-2 fluorescence microscope with a MagnaFire® digital microscope camera (Optronics®) and Image-Pro Plus software (Media Cybernetics).

Results and Discussion

Cell Culture Surfaces

Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide and Corning PureCoat ECM Mimetic Cultureware Collagen I peptide surfaces are synthetic, xeno-free, and animal-free cell culture surfaces containing covalently-attached Fibronectin- and Collagen I-based peptides, respectively. Collagen I peptide consists of a GFOGER amino acid sequence that facilitates attachment of α2 integrin positive cells, and the Fibronectin peptide consists of an RGD-based amino acid sequence to support attachment of cells expressing α5 integrin. These surfaces present the peptide fragments in a functional manner providing a unique culture environment that promotes cell attachment and proliferation, in a room temperature stable and ready-to-use format.

Cell Culture and Passaging

ECFCs were cultured on Collagen I mimetic and Fibronectin mimetic surfaces for 4 passages. Population doubling was calculated based on cell yield. Cells grown on ECM mimetic surfaces grew faster than those grown on self-coated native ECM surfaces, as exhibited by an increase in cumulative population doublings (Figure 1a). Cell morphology was also comparable on the ECM mimetic surfaces and native protein-coated surfaces (Figure 1b).

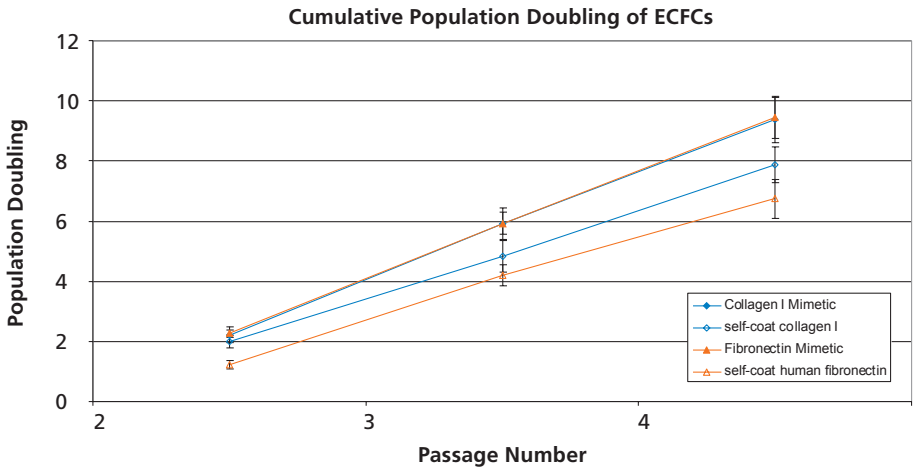
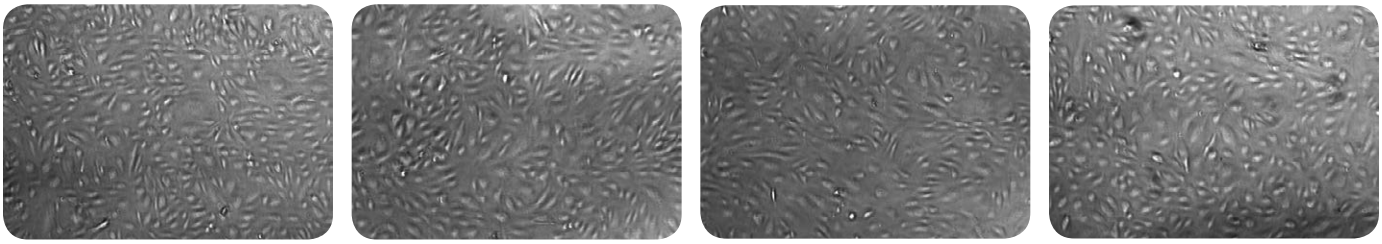


Figure 1a. Population doubling curves of ECFCs cultured on ECM Mimetic surfaces versus native ECM-coated surfaces.

Figure 1b. ECFCs on Corning PureCoat Collagen I and Fibronectin mimetic surfaces and native protein-coated surfaces. Cells exhibited comparable morphology.

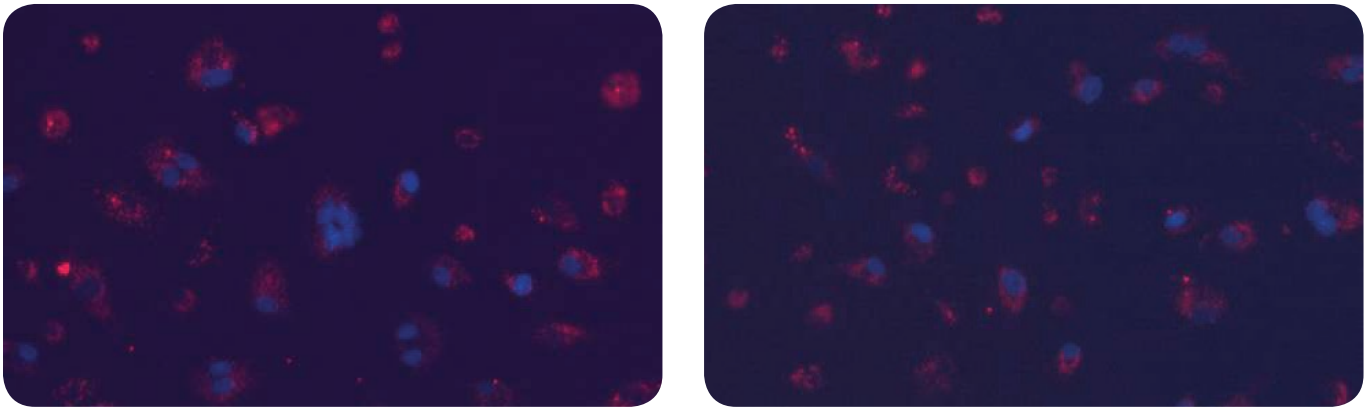


Corning PureCoat Collagen I mimetic surface | **Corning PureCoat Fibronectin mimetic surface** | **Rat tail Collagen I self-coated surface** | **Human Fibronectin self-coated surface**

Functional Assays - Cell Staining and Imaging

After three passages on Collagen I mimetic and Fibronectin mimetic surfaces, DiI AcLDL was added to the cells and uptake was monitored by fluorescence imaging. Cells were able to uniformly incorporate DiI AcLDL (Figure 2), demonstrating that ECFCs maintained their endothelial lineage when cultured on the ECM mimetic surfaces.

Figure 2. DiI AcLDL uptake (red) by ECFCs; nuclei were stained with DAPI (blue).

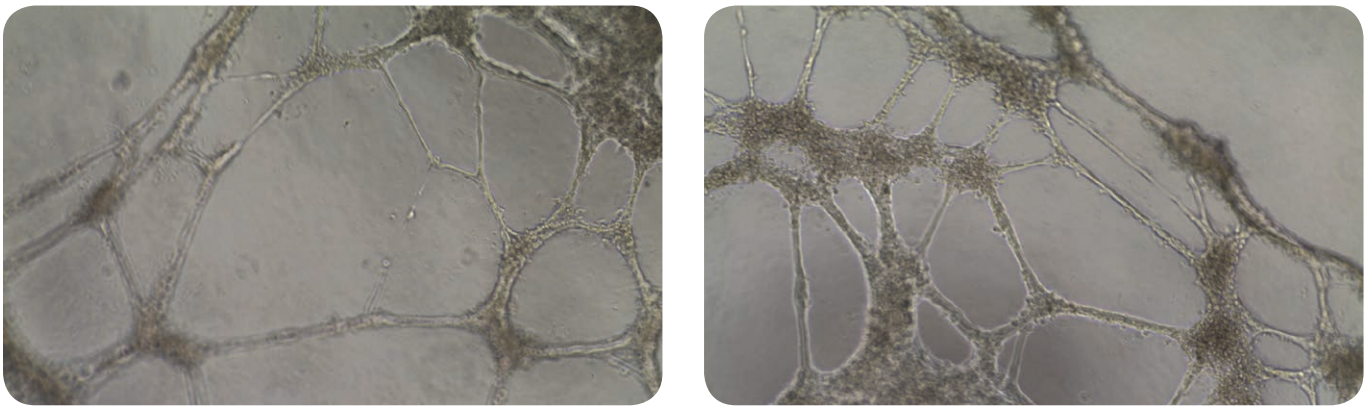


Corning PureCoat Collagen I mimetic surface | **Corning PureCoat Fibronectin mimetic surface**

Functional Assays - Tube Formation

Following three passages on ECM mimetic surfaces, functionality of ECFCs was assessed by the tube formation assay, a characteristic feature of endothelial cells. ECFCs were able to form capillary tube-like structures upon overnight incubation on Corning Matrigel® matrix (Figure 3), demonstrating post-expansion functionality on the ECM mimetic surfaces.

Figure 3. Capillary tube-like structures formed after plating adherent ECFCs on Corning Matrigel matrix.



ECFCs from Corning PureCoat Collagen I mimetic surface | **ECFCs from Corning PureCoat Fibronectin mimetic surface**

Conclusions

- Corning PureCoat ECM Mimetic Cultureware are animal-free, synthetic, ready-to-use culture vessels that consist of covalently-immobilized cell-adhesion promoting peptides derived from Fibronectin and Collagen I.
- Corning PureCoat ECM Mimetic Cultureware successfully supported culture of endothelial colony forming cells as well as, or better than, native-ECM-coated surfaces. These cells maintained functionality when cultured on the ECM mimetic surfaces for multiple passages.

References

1. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104:2752–2760 (2004).
2. Thomas RA, Pietrzak DC, Scicchitano MS, Thomas HC, McFarland DC, Frazier KS. Detection and characterization of circulating endothelial progenitor cells in normal rat blood. *Journal of Pharmacological and Toxicological Methods* 60:263–274 (2009).
3. Napoli C, Hayashi T, Cacciatore F, Casamassimi A, Casini C, Al-Omran M, Ignarro LJ. Endothelial progenitor cells as therapeutic agents in the microcirculation: An update. *Atherosclerosis* 215:9–22 (2011).
4. Hristov M, Weber C. Endothelial progenitor cells: Cellular biomarkers in vascular disease.*Drug Discovery Today: Disease Mechanisms* 5:e267–271 (2008).
5. Lu S-J, Feng Q, Caballero S, Chen Y, Moore MAS, Grant MB, Lanza R. Generation of functional hemangioblasts from human embryonic stem cells. *Nature Methods* 4:501–509 (2007).
6. Voyta JC, Via DP, Butterfield CE, Zetter BR. Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. *Journal of Cell Biology* 99:2034–2040 (1984).
7. Körbling M, Reuben JM, Gao H, Lee B, Harris DM, Cogdell D, Giralt SA, Khouri IF, Saliba RM, Champlin RE, Zhang W, Estrov Z. Recombinant human granulocyte–colony-stimulating factor–mobilized and apheresis-collected endothelial progenitor cells: a novel blood cell component for therapeutic vasculogenesis. *Transfusion* 46:1795–1802 (2006).
8. Fadini GP, Baesso I, Albiero M, Sartore S, Agostini C, Avogaro A. Technical notes on endothelial progenitor cells: Ways to escape from the knowledge plateau. *Atherosclerosis* 197:496–503 (2008).
9. DeGroot K, Bahlmann FH, Sowa J, Koenig J, Menne J, Haller H, Fliser D. Uremia causes endothelial progenitor cell deficiency. *Kidney International* 66:641–646 (2004).
10. Hur J, Yoon C-H, Kim H-S, Choi J-H, Kang H-J, Hwang K-K, Oh B-H. Lee M-M, Park Y-B. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol* 24:288–293 (2004).

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