

Cell Growth and Differentiation

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Enhancing Cell Culture and Accelerating Discovery

The development and normal functioning of cells depends on interactions with molecules in their microenvironment. The major classes of molecules that regulate cellular development and function include growth and differentiation factors, cell adhesion molecules, and the components of the extracellular matrix (ECM). The ECM, composed of a number of different

macromolecules, influences behavior, (adherence, spreading, differentiation, and migration) and the pattern of gene expression of the cells in contact with it. To create physiologically relevant *in vitro* models that support normal cell growth and function, the components of the *in vivo* environment must be incorporated. Use of ECM proteins as coating for tissue culture surfaces permits the development of cell type specific model systems which closely mimic *in vivo* conditions.

Recognizing the increasingly important role the ECM plays in the regulation of fundamental cellular processes Corning offers a wide range of extracellular matrix proteins and attachment factors for researchers to incorporate into their cell culture systems. For over 20 years, we have provided the research market with a wide variety of purified proteins. We were the first to offer a unique line of tissue culture vessels coated with a variety of ECM proteins and attachment factors: Corning® BioCoat™ Cellware. Our extensive experience in protein purification, along with rigorous quality assurance testing guarantees high-quality, consistent products.

At Corning we are committed to enhancing cell culture and accelerating discovery worldwide through dedicated customer service, innovative product solutions, and technical expertise. We strive to make cell culture research more efficient and convenient for researchers by offering outstanding quality, consistency, and value.

Commitment to Quality

We understand the importance of lot-to-lot consistency and the need for reproducible results. Through proprietary manufacturing technology, validated procedures, strict compliance with established protocols, and exacting quality control, we are able to assure the biological performance of our products as well as consistency from lot-to-lot.

Delivering Choice

The optimal surface for cell attachment, proliferation, and differentiation is dependent on the particular cell type. Falcon®, Corning BioCoat, and Corning ECM proteins provide diverse options for a variety of cells, including but not limited to commonly used cell lines such as HEK-293, primary neuronal cells, and threedimensional culture.

Technical Expertise

Our scientists routinely study a broad range of cells to better understand their cellular function. Our team of highly skilled and dedicated Technical Support Specialists are available to assist you in protocol development and troubleshooting.

Customizable Solutions

We offer a custom product service to meet the unique needs of our customers. Our custom capabilities range from special package sizes and sterilization needs to barcoding and custom coating. Through our custom coating services, we will apply the coating of your choice on Corning and alternative cultureware products. If you are not sure which coating you need, our Technical Support Specialists can recommend surfaces for your cell type.

Cell Culture Surfaces

Corning offers a wide variety of surface chemistries and attachment factors appropriate for a broad range of applications. The surface of our Falcon® Cultureware is rendered permanently hydrophilic via a unique vacuum-gas plasma tissue culture treatment process. This treatment process is produced in a closed, highly controlled environment ensuring a consistent treatment surface. Corning® Primaria™ and Corning BioCoat™ surface options are ideal for enhanced cell attachment and growth of a variety of primary cells, stem cells, and transformed cell lines in serum-free or serum-containing cultures. Corning PureCoat™ surfaces are a novel family of chemically synthesized and animal-free surfaces that enhance cell attachment and growth in low-serum or serum-free culture environments. A non-treated surface is also available for suspension or non-adherent cell culture and may also be used to study cell-cell or cell-protein interactions in an *in vitro* system.

Falcon Non-treated Polystyrene

• Hydrophobic surface with low to moderate binding properties. Ideal for cell-cell or cell-protein studies.

Falcon Tissue Culture-treated (TC)

- • Hydrophilic surface enhances cell attachment, spreading, and cell growth by binding serum proteins to the surface. Highly controlled vacuum-gas plasma treatment creates negatively charged carboxyl groups on the polystyrene surface.
- Tested for confluency of MRC-5 cells and sterilized by gamma-irradiation.

Corning Primaria

- Supports neuronal, primary, endothelial, and tumor cells which may have difficulty attaching to or differentiate poorly on traditional TC surfaces. This surface has a unique mixture of negative and nitrogen containing positive functional groups on the polystyrene surface.
- The surface consistency of each lot is confirmed by electron spectroscopy chemical analysis (ESCA).

Corning BioCoat Poly-D-Lysine (PDL)

- Pre-coated with PDL, which promotes cell attachment of transfected and primary cells (e.g., neuronal).
- Tested for the ability to promote firm attachment of rat cerebellar granule (RCG) cells.
- Stable for six months from date of shipment at 4-30°C. Coverslips, CultureSlides, and Coverslip-Bottom Dishes stable for at least three months from date of shipment at 4°C.

Corning BioCoat Collagen I

- Pre-coated with Collagen I, derived from rat tail tendon.
- Tested for the ability to promote attachment and spreading of HT-1080 human fibrosarcoma cells.
- Stable for at least six months from date of shipment when stored at 4-30°C under dry conditions. Coverslips and CultureSlides are stable for at least three months from date of shipment when stored at 2-8°C.

Corning BioCoat Collagen IV

- Pre-coated with Collagen IV. Useful as a substrate for nerve, epithelial, endothelial, and muscle cells.
- Tested for the ability to promote attachment and spreading of PC12 rat pheochromocytoma cells or to initiate differentiation (neurite outgrowth) of NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

Corning BioCoat Gelatin

- Pre-coated with Gelatin, which is commonly used for culture of vascular endothelial cells and F9 teratocarcinoma cells.
- Tested to promote proliferation of Human Umbilical Vein Endothelial Cells (HUVEC).
- Stable for at least three months from date of shipment when stored at 4-30°C under dry conditions.

Corning BioCoat Fibronectin

- Pre-coated with Human Fibronectin (HFN), which promotes cell attachment through integrin binding. HFN promotes cellular migration during wound healing and improves survival of primary cells.
- Tested to promote attachment and spreading of BHK-1 hamster kidney cells.
- Stable for at least three months at 2-8°C. Do not freeze.

Corning BioCoat Laminin

- Pre-coated with Laminin, a major component of the basement membrane used as a substrate to culture and maintain differentiated functions of a variety of cells including neuroblastoma cells and breast cancer cell lines.
- Tested for the ability to initiate neurite outgrowth of NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

Corning BioCoat Laminin/Fibronectin

- Pre-coated with a combination of ECMs, which provide superior attachment and growth of glial precursor cells.
- Tested for receptor agonist induced changes in intracellular calcium-using FLUO-3 in primary rat cortical enriched cultures.
- Stable for at least three months at 2-8°C. Do not freeze.

Corning BioCoat Poly-D-Lysine/Laminin (PDL/Laminin)

- Pre-coated with a combination of ECMs, which supports neuronal differentiation of human and mouse stem cells.
- Tested for the ability to promote neurite outgrowth with primary rat cerebellar granule (RCG) cells and NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least 3 months at 2-8°C. Do not freeze.

Corning BioCoat Poly-L-Ornithine/Laminin (PLO/Laminin)

- Pre-coated with a combination of ECMs, which support growth of neuroblastoma cells and differentiation of N2a and ScN3a cells.
- Tested for the ability to promote neurite outgrowth with primary rat cerebellar granule (RCG) cells and NG-108 rat glioma/mouse neuroblastoma cells.
- Stable for at least three months at 2-8°C. Do not freeze.

Corning BioCoat Matrigel® Matrix

- Pre-coated with solubilized basement membrane matrix extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma. Rich in ECM proteins, especially laminin, collagen IV, heparin sulphate proteoglycans, and entactin.
- Tested for the ability to promote neurite outgrowth from chick dorsal root ganglia in the absence of Nerve Growth Factor (NGF).
- Stable for at least three months at -20°C. Keep frozen until use.

Corning PureCoat ECM Mimetic Fibronectin Peptide

- Consists of RGD sequences to support the attachment of cell types that require Fibronectin coating including alpha-5 integrin-positive cells.
- Compatible, animal-free alternative to natural animal or human ECM surfaces, such as natural human Fibronectin for hMSC expansion and differentiation.

Corning PureCoat ECM Mimetic Collagen I Peptide

- Supports the attachment of Collagen I-dependent cell types including alpha 2 integrin-positive cells (and others).
- Compatible, animal-free alternative to natural animal or human ECM surfaces, such as natural human Collagen I for human keratinocyte expansion.

PRODUCT SELECTION BY CELL TYPE

For guideline use only. This is not a complete list of all applications for these products.

www.corning.com/lifesciences | 3

Human Embryonic Stem Cells

Human embryonic stem (hES) cells are pluripotent cells derived from the inner cell mass of a blastocyst. These cells can either self-renew, thereby maintaining their pluripotency, or differentiate into all three germ layers depending upon the culture conditions. Induced pluripotent stem (iPS) cells, which are similar in potential to hES cells, have been generated by infecting adult cells. iPS cells, like hES cells, can form all three germ layers as well as self-renew. Tremendous hope is associated with the potential application of hES and iPS cells in cell therapy and regenerative medicine because of their ability to differentiate into multiple, clinically useful cell types. Defined culture conditions are essential to realizing the potential of hES and iPS cells.

A culture environment for hES cells consisting of both a serum-free, defined medium, and a cell culture surface specifically qualified for hES cells saves researchers time and resources normally spent qualifying reagents. Corning® Matrigel® Matrix, coupled with a variety of culture media, has been widely accepted as an alternative substrate to feeder-dependent culture of hES cells¹⁻⁴, and Corning Matrigel Matrix has been used to culture iPS cells⁵⁻⁶. Corning Matrigel Matrix is a reconstituted basement membrane isolated from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma.

STEMCELL Technologies has commercially developed and optimized WiCell™ Research Institute's mTeSR®1 medium formulation to standardize feeder-independent hES cell culture. mTeSR1 is complete, defined and serum-free, and has been designed to

FIGURE 1 • HUMAN EMBRYONIC STEM CELLS CULTURED ON CORNING MATRIGEL hESC-QUALIFIED MATRIX

1A. Phase contrast images of H9 colonies grown on mouse embryonic fibroblast (MEF) feeder layer in hES media (left), Corning Matrigel hESC-qualified Matrix in MEF-conditioned media (middle), or mTeSR®1 maintenance media (right). Images were taken at 4x magnification.

1B. Flow cytometry analysis of H9 cells cultured on Corning Matrigel hESC-qualified Matrix coated surface in mTeSR1 maintenance media. Cells were probed with the following antibodies: Tra-1-60 PE (Cat. No. 560193), Tra-1-81 PE (Cat. No. 560161), SSEA-4 PE (Cat. No. 560128) and Oct3/4 PE (Cat. No. 560186) compared to isotype control. Percent positive is indicated. Cells were run on a BD FACSCalibur™ system and the data was analyzed with BD CellQuest™ software.

maintain and expand hES cells in an undifferentiated state when used with Corning Matrigel® hESC-qualified Matrix as a substrate (**Figure 1**).

An alternative surface for hES cell culture is Corning Laminin/Entactin Complex High Concentration (**Figure 2**). Corning Laminin/Entactin Complex High Concentration, with a purity greater than or equal to 90%, is a more defined surface that can support undifferentiated hES cell growth. Unlike Corning Matrigel hESC-qualified Matrix, this surface is not specifically qualified for maintenance of undifferentiated hES cells.

2A. Phase contrast images of H9 cells grown on Corning Matrigel hESC-qualified Matrix (left) and Corning Laminin/Entactin Complex High Concentration (right) in mTeSR1 maintenance media. Images were taken at 4x magnification.

2B. Flow cytometry analysis of H9 cells cultured on Corning Laminin/Entactin Complex High Concentration (red line) and Corning Matrigel hESC-qualified Matrix coated surface (green line) in mTeSR1 maintenance media. Cells were probed with the following antibodies: SSEA-4 PE (Cat. No. 560128) and Oct3/4 PE (Cat. No. 560186) compared to isotype control (black line). Cells were run on a BD FACSCalibur™ system and the data was analyzed with BD CellQuest™ software. Both surfaces supported undifferentiated expansion of hESC, H9.

2C. G banding chromosome analysis. Karyotype analysis of H9 cells grown on Corning Laminin/Entactin Complex High Concentration in mTeSR1 media for 26 passages. Cells maintained normal karyotype under these culture conditions.

Tools for Human Embryonic Stem Cell Culture

For a complete product listing, see page 19.

Tissue Culture-treated

DID YOU KNOW?

• Corning offers a full range of pipets and tubes. Please contact your sales representative for more information.

Endothelial Cells

Endothelial cells are a specialized type of epithelial cell which forms the inner layer of blood vessels. These cells play a key role in angiogenesis, the development of new blood vessels from pre-existing vessels. Angiogenesis is a multi-step process that is important for both physiological and pathological development. During angiogenesis, endothelial cells are activated and express matrix metalloproteinases (MMPs), which degrade the vascular basement membrane. In response to environmental cues, endothelial cells secrete MMPs and then invade through the basement membrane to form new capillary networks.

Endothelial cells are tested in a variety of assays for functions that contribute to the angiogenesis process. Collagen I coated surfaces are suitable for culturing endothelial cells such as fetal bovine heart endothelial cells (FBHECs) and human umbilical vein endothelial cells (HUVECs) (**Figure 3**). *In vitro* assays of endothelial cell function include cell migration⁷, invasion⁸, and tubule formation⁹⁻¹⁵. Both the Corning® BioCoat™ Angiogenesis System: Endothelial Cell Invasion and the Corning BioCoat Angiogenesis System: Endothelial Cell Migration allow for rapid data collection without multiple handling steps. These quantitative assays utilize Corning FluoroBlok™ microporous polyethylene terephthalate (PET) membranes (3 µm pore size) which effectively block the fluorescence signal from labeled cells that have not invaded or migrated through the membrane, respectively, thereby allowing the selective detection of cells that reside on the underside of the membrane (**Figure 4**). To perform fluorescence detection, cells may be pre-labeled or post-labeled with a fluorescent dye (**Figure 5**). The pre-labeling technique enables real-time kinetic measurements of cell migration or invasion. Endothelial cells must be able to migrate and enzymatically degrade the basement membrane in order for angiogenesis to occur. The wells of Corning® BioCoat Angiogenesis System: Endothelial Cell Invasion are evenly coated with Corning Matrigel® Matrix, which allows researchers to examine the ability of endothelial cells to invade through reconstituted basement membrane in response to chemoattractants, such as VEGF, in the presence or absence of anti-angiogenic agents (**Figure 6**).

FIGURE 3 • EFFECTS OF CORNING BIOCOAT ENDOTHELIAL CELL GROWTH ENVIRONMENT ON HUVEC

Corning BioCoat Endothelial Cell Growth Environment utilizes Corning BioCoat Collagen I Cellware and Corning Endothelial Cell Culture Medium to enhance endothelial attachment and proliferation. HUVECs grown for five days using the Corning BioCoat Endothelial Cell Growth Environment form a confluent monolayer and show numerous mitotic cells (A). HUVECs grown for five days in basal medium containing 10% FBS on tissue culture-treated plastic show sparse growth (B).

The use of Corning Cell Recovery Solution or Corning Dispase is necessary to recover cells cultured on Corning Matrigel Matrix.

FIGURE 4 • LABELING CELLS POST-INVASION WITH CALCEIN AM

A fluorescence plate reader quantifies cells post-invasion by measuring fluorescence which correlates to cell number. Cells on top of the Corning® FluoroBlok™ membrane are not detected by a bottom-reading fluorometer.

FIGURE 5 • LABELING METHODS FOR ENDPOINT OR REAL-TIME KINETIC MIGRATION AND INVASION ASSAYS

Corning FluoroBlok Inserts can be used for endpoint or real-time kinetic assays. For endpoint assays, the cell migration or invasion assay is performed with unlabeled cells. .
At the end of the assay the cells are labeled with a fluorescent dye, such as Corning Calcein AM, and the data is collected using a bottom reading fluorescent plate reader. For real-time kinetic assays, the cells are pre-labeled with a fluorescent dye, such as Corning DiIC $_{12}(3)$. After labeling, the migration or invasion assay is run with data collected over a time course using a bottom reading fluorescent plate reader.

FIGURE 6 • EFFECTS OF TIMP-2 AND 1'10' PHENATHANTHROLINE IN VEGF-MEDIATED HMVEC INVASION

Human microvascular endothelial cells (HMVECs) were assayed in the Corning BioCoat™ Angiogenesis System: Endothelial Cell Invasion in the presence of VEGF (4 µg/mL) with varying concentrations of (left) TIMP-2 or (right) 1'10' phenanthroline in the bottom chamber. Cells were allowed to invade for 22 \pm 1 hour. Cells were labeled post-invasion with Corning Calcein AM (4 µg/mL) and then analyzed for invasion through Corning Matrigel® Matrix using an Applied Biosystems CytoFluor® 4000 plate reader [485/540 nm (Ex/Em) wavelengths]. Data represents the mean of $n=3$ inserts \pm S.D.

Tools for Endothelial Cell Culture

Cat. No. Description Qty. Cell Cu

Extracellular Matrix Proteins

Cell Recovery Reagents

Fluorescent Dyes

Culture Media **Cytokines and Media Additives**

Cell Culure Tools

Corning BioCoat Collagen I Cellware

Cell Environments

Corning BioCoat Cell Environment

Membrane Insert Systems

Corning BioCoat Angiogenesis System: Endothelial Cell Migration

354143 24-Multiwell Insert Plate with lid 1

Corning BioCoat Angiogenesis System: Endothelial Cell Invasion

354141 24-Multiwell Insert Plate with lid 1

Corning BioCoat Angiogenesis System: Endothelial Tube Formation

354149 96-Multiwell Insert Plate with lid 1

For a complete product listing, see page 19.

Corning® BioCoat™ Angiogenesis System: Endothelial Cell Migration consists of Corning FluoroBlok™ inserts evenly coated with human fibronectin (**Figure 7**). Studies conducted using the post-labeling technique demonstrated that Corning HUVEC-2 cells migrate towards VEGF in a concentration dependent manner (**Figure 8**).

During angiogenesis, endothelial cells form capillaries once they have invaded through the basement membrane. The correct culture surface is critical for successful endothelial cell tube formation *in vitro*.

* Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation offers a standardized and robust assay for studying endothelial cell tubulogenesis.
For customers interested in establishing an assay for tube formatio optimal performance.

FIGURE 7 • HUVEC MIGRATION ON UNCOATED AND HUMAN FIBRONECTIN-COATED INSERTS

Migration assays were conducted using HUVECs in the Corning BioCoat Angiogenesis System: Endothelial Cell Migration and compared with uncoated Corning FluoroBlok 24-Multiwell Inserts using both FBS (5%) and VEGF (10 µg/ mL) as chemoattractants. The cells were allowed to migrate for 22 \pm 1 hour. Cells were labeled post-migration with Calcein AM (4 µg/mL) and measured by detecting the fluorescence of the cells that migrated through the Corning FluoroBlok membrane using an Applied Biosystems CytoFluor® 4000 plate reader [485/530 nm (Ex/ Em) wavelengths]. The results indicate a marked increase in migration in response to VEGF when the assay was performed on the fibronectin-coated inserts included in the system. Data represents the mean of $n=3$ inserts \pm S.D.

FIGURE 8 • CORNING HUVEC-2 CELLS EXHIBIT CONCENTRATION-DEPENDENT MIGRATION TOWARDS VEGF

Corning HUVEC-2 cells assayed in the Corning BioCoat Angiogenesis System: Endothelial Cell Migration (96-Multiwell format) in response to increasing concentrations of VEGF. Samples were incubated for 22 hours. Cells were labeled post-migration with Corning Calcein AM and measured by detecting the fluorescence of cells that migrated through the fibronectin-coated Corning FluoroBlok membrane with the Victor2™ plate reader (PerkinElmer) at 485 nm emission. Data represents the mean of $n=4$ inserts \pm S.D.

Both primary endothelial cells and endothelial cell lines have been demonstrated to form tubules on the Corning® BioCoat™ Angiogenesis System: Endothelial Cell Tube Formation (**Figures 9-11**) which is comprised of a 3D gel of Corning Matrigel® Matrix. The Corning BioCoat Angiogenesis Systems are available in 24 and 96-Multiwell formats, which can be used for moderate to high throughput compound screening. Corning Matrigel Matrix has also been extensively used to study *in vivo* angiogenesis^{10-11, 16-18 as a less technically challenging alternative to the} corneal implantation model. A "plug" of material is placed subcutaneously, followed by histological quantification 7-10 days later. These *in vitro* and *in vivo* assays give researchers multiple options for exploring endothelial cell functions that are essential during angiogenesis.

FIGURE 9 • HUMAN ENDOTHELIAL CELL TYPES EXHIBIT TUBE FORMATION

HUVEC, HMVEC, and the human endothelial cell line HMEC-1 exhibit tube formation on Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. For this study, 20,000 cells of each cell type were added to wells containing presolidified Corning Matrigel Matrix. The assay was incubated for 18 hours. Each bar represents the mean of $n=32$ wells \pm S.D.

FIGURE 10 • CONFOCAL IMAGE OF CORNING HUVEC-2 CELL TUBE FORMATION

Corning HUVEC-2 cells were assayed using the Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. Cells were stained using Corning Calcein AM. Confocal images were captured using the BD Pathway™ Bioimager in confocal mode using the 4x objective (NA 0.13) for quantification of tubule formation.

FIGURE 11 • SURAMIN INHIBITS HMEC-1 TUBE FORMATION

HMEC-1 cells (40,000 cells/mL) were treated with Suramin at concentrations ranging from 0-40 µm and then analyzed for tube formation using Corning BioCoat Angiogenesis System: Endothelial Cell Tube Formation. 50 µl of cells plus compound were added to wells containing presolidified Corning Matrigel Matrix. Samples were incubated at 37°C, 5% CO₂ for 18 hours before staining with Corning Calcein AM. Images were acquired with a 2x objective lens and the total tube length was measured using MetaMorph® (Universal Imaging Corporation™). Each bar represents the mean of $n=8$ wells \pm S.D.

Pseudo-colored image for illustrative purposes only.

Hepatocytes

Hepatocytes are liver epithelial cells used for both basic research and drug metabolism studies. Fresh and cryopreserved primary hepatocytes contain all the major enzyme pathways for drug and xenobiotic biotransformation. These include the major phase I drug metabolism enzyme family (P450) and phase II enzymes (UGT, SULT, GST and NAT). Hepatocytes also contain all the gene regulation pathways for P450 induction. Appropriate culture conditions are required to maintain hepatic P450 activity.

Hepatocytes can be cultured on Collagen I¹⁹⁻²², Corning[®] Matrigel[®] Matrix²³⁻²⁷ or Corning PuraMatrix™28-29. Corning BioCoat™ Collagen I Cellware is a commonly used surface for cultures of both fresh and cryopreserved hepatocytes³⁰⁻³¹ (Figure 12). Cells cultured on this surface maintain their biological activity, as shown by P450 induction (**Figure 13**). Sandwich cultures, such as hepatocytes grown on Corning BioCoat Collagen I with Corning Matrigel Matrix overlay, are used to assess bile canaliculi formation³². Choly-lysyl-fluorescein (CLF) is a fluorescein-labeled bile acid that is secreted into bile canaliculi by ABC efflux transporters which can be used to visualize bile canaliculi (**Figure 14**). BD Matrigel Matrix has been shown to suppress cell growth and prevent growth-associated dedifferentiation²³, as well as maintain liverspecific functions *in vitro* longer than most collagen-based systems²⁴⁻²⁶. Hepatocytes cultured on Corning Matrigel Matrix also have a more differentiated morphology than hepatocytes cultured on collagen I (**Figure 15**). Both Corning Collagen I and Corning Matrigel Matrix are animal-derived products; Corning PuraMatrix, a synthetic peptide hydrogel, is a suitable alternative for assays that require a xeno-free culture environment. Therefore, the appropriate culture surface depends on the experimental goals (e.g., drug metabolism, bile canaliculi formation or xeno-free environment).

FIGURE 12 • CORNING INDUCIBLE CRYOPRESERVED HUMAN HEPATOCYTES CULTURED ON CORNING BIOCOAT COLLAGEN I

Corning Gentest™ Inducible-qualified Human CryoHepatocytes were isolated using the Corning Gentest CryoHepatocyte Purification Kit and resuspended in freshly prepared ISOMs seeding media at a concentration of 1x10⁶ cells/ mL. Cells were plated onto Corning BioCoat Collagen I 24-well plates and incubated for approxiamately 2 hours, after which plating media was removed and replaced with supplemented Corning Hepatocyte Culture Media.

• Corning offers a custom barcoding service. This service provides highquality barcode labels affixed to any side of a microplate.

FIGURE 13 • INDUCTION OF CORNING GENTEST™ INDUCIBLE-QUALIFIED HUMAN CRYOHEPATOCYTES

Corning Gentest Inducible-qualified Human CryoHepatocytes were isolated using the Corning Gentest CryoHepatocyte Purification Kit and resuspended into freshly prepared ISOMs seeding media at a concentration of 1x10⁶ cells/mL. Cells were plated onto Corning BioCoat™ Collagen I 24-well Multiwell Plates and incubated for approximately 2 hours, after which plating media was removed and replaced with supplemented Corning Hepatocyte Culture Media. Cells were monitored for degree of attachment at 18-24 hours after plating and daily during the experiment. Cells were induced with either 20 µM Rafampicin (A) or 20 µM β-Napthoflavone (B) over a 3-day period. Controls were treated with the appropriate solvent control. Metabolic activity was determined on day 5 of the experiment using 200 µM Testosterone as a substrate to measure CYP3A4 activity and 100 µM Phenacetin as a substrate for CYP1A2. Assays were run for 30 minutes and 60 minutes, respectively. Analysis was performed by HPLC and activity expressed per mg of protein.

FIGURE 14 • CORNING GENTEST CHOLY-LYSYL-FLUORESCEIN SEQUESTERED IN BILE CANALICULI

CLF sequestered in the bile canaliculi of Corning Gentest Inducible-qualified Human CryoHepatocytes cultured on Corning BioCoat Collagen I overlaid with Corning Matrigel Matrix.

FIGURE 15 • EFFECTS OF ECM ON CELL MORPHOLOGY: MICROGRAPHS OF HEPATOCYTES CULTURED ON VARIOUS CULTURE SUBSTRATA

Scanning electron micrographs of primary rat hepatocytes cultured for two days on Collagen I (A), Collagen I gel (B), or Corning Matrigel Matrix (C). Note the clusters of spherical cells for hepatocytes cultured on Corning Matrigel Matrix, typical of differentiated cells.

Tools for Hepatocyte Cell Culture

For a complete product listing, see page 19.

Neuronal Cells

Neuroscience is a rapidly evolving field that encompasses a variety of cell types, including neurons and neuronal stem cells. *In vitro* culture of these diverse cell types requires appropriate culture surfaces for attachment and proliferation/ differentiation, as detailed in the examples below. NG-108 rat glioma/mouse neuroblastoma cells and PC-12 cells, two neuronal cell lines, require different surfaces for attachment. NG-108 cells attach loosely to tissue culture-treated cellware, but when they are cultured on Corning® BioCoat™ Laminin Cellware they exhibit a more typical neuronal morphology (**Figure 16**). PC-12 cells, derived from a transplantable rat pheochromocytoma, develop neurites in response to NGF when they are cultured on collagen I (**Figure 17**). Other surfaces, including Corning BioCoat Poly-D-Lysine Cellware³³ and Corning BioCoat Poly-D-Lysine/Laminin³⁴, can also be used to culture PC-12 cells. Primary neuronal cells utilize different attachment surfaces depending on their origin and the composition of the media used during culture. Primary mouse cortical neurons and primary mouse basal forebrain cholinergic neurons have been cultured on Corning BioCoat Poly-L-Lysine Cellware³⁵ and Corning BioCoat Poly-D-Lysine/Laminin Cellware³⁶, respectively. Primary human neural stem cells have been grown under serum-containing conditions in tissue culture-treated Corning Falcon® Cell Culture Flasks³⁷. Using serum-free conditions, Thonhoff, et al., showed that neuronal stem cells maintain their capacity to differentiate into both Tuj1+ neuronal cells and GFAP+ astroglial cells on Corning PuraMatrix™ while differentiation of neuronal stem cells grown on Corning Matrigel® Matrix was skewed toward GFAP+ astroglial cells³⁸. Both Corning PuraMatrix³⁸⁻⁴⁰ and Corning Primaria™41 are defined, xeno-free surfaces for 3D and 2D culture, respectively, which are compatible with neuronal cells. Corning Primaria Cultureware enhances neuronal cell attachment as compared to tissue culture-treated cellware, as shown with chick embryo spinal cord neurons (**Figure 18**). These examples* illustrate the need for an appropriate growth surface which is determined by the cell type and whether a xeno-free surface with defined media is required by the experimental model.

*Other examples available in references 42-44.

FIGURE 16 • EFFECTS OF CORNING BIOCOAT LAMININ CELLWARE ON NG-108 NEUROBLASTOMA CELLS

NG-108 rat glioma/mouse neuroblastoma cell morphology is surface dependent. Cells cultured on tissue culture plastic are loosely adhered and remain rounded (A). Cells cultured on Corning BioCoat Laminin cellware exhibit a spindle-shaped morphology and dendritic processes (B).

Corning offers a full range of 96-, 384-, and 1536-well Microplates. Custom packaging, labeling (e.g., barcoding), and custom coatings are also available. Please contact your sales representative for more information.

FIGURE 17 • PC12 NEURITE OUTGROWTH, CULTURED ON CORNING® COLLAGEN I

PC12 cells were maintained in DMEM with 10% FBS, 5% horse serum and 1% penicillin/ streptomycin. For neurite generation, approximately 15,000 cells/well were plated in Falcon® 96-well plates that were coated with Corning Collagen I, rat tail using 1.8 µg collagen per well. After 24 hours, the medium was replaced with differentiation medium (DMEM with 0.1% FBS, 0.05% horse serum, 100 ng/mL NGF). The medium was replenished every third day for 10 days. For imaging, cells were fixed with 3.7% paraformaldehyde for 20 minutes and permeabilized with 0.1% Triton-X-100 for 5 minutes. Neurites were stained with a primary mouse anti-β-tubulin antibody (Cat. No. 556321) using 0.125 µg antibody/well followed by AlexaFluor® 488 goat anti-mouse IgM at a concentration of 0.25 µg/well. Hoechst 33342 was used at 0.1 µg/well to stain the nuclei. To prevent the dissociation and fracture of fragile neuronal networks, the number of washes in the fixation and processing steps were minimized and extra care was taken in aspirating and dispensing liquids in wells. Images were acquired on a BD Pathway™ as a 4x4 montage using a 20x objective (0.75 NA).

FIGURE 18 • CHICK EMBRYO SPINAL CORD NEURONS CULTURED ON CORNING PRIMARIA™ CULTUREWARE

When chick embryo spinal cord neurons are cultured on Corning Primaria™ Cultureware, growth is enhanced and extensive neurite development occurs. In this experiment, cells clumped and detached from traditional TC plates after 20 days in culture (A) but remained viable and differentiated on Corning Primaria Cultureware (B).

Tools for Neuronal Cell Culture

Cat. No. Description Qty. Cell Culture Reagents

Extracellular Matrix Proteins

Cytokines and Media Additives

Cell Recovery Reagents

Cell Culture Tools

For a complete product listing, see page 19.

FIGURE 19 • PROLIFERATION OF HUMAN NEONATAL KERATINOCYTES ON CORNING BIOCOAT™ COLLAGEN I

Human neonatal keratinocytes cultured on Corning BioCoat Collagen I.

Corning offers custom coatings. Please contact your sales representative for more information.

Epithelial Cells

Epithelial cells are found throughout the body, from skin to glandular formations within tissues. *In vivo* these cells are attached to a three dimensional basement membrane matrix. The interactions between the epithelial cell and matrix proteins effect cell morphology and function. Two highly specified epithelial cell types have been discussed in the hepatocyte and endothelial cell sections, utilizing both 2-dimensional (2D) and three-dimensional (3D) culture systems. *In vitro*, 2D and 3D culture systems can be used to study different aspects of cell growth and differentiation. 2D culture systems are used for cell attachment and proliferation. 3D environments are utilized in studies requiring a more *in vivo*-like setting, such as mammary acini formation.

The Corning® BioCoat™ Cellware provides a range of 2D surfaces for cell growth. Both keratinocytes⁴⁵⁻⁴⁶ and HEK-293⁴⁷⁻⁴⁹ cells are examples of epithelial cells that can be studied in 2D culture environments. Keratinocytes are a major component of the epidermis; Corning BioCoat Collagen I supports growth of human neonatal keratinocytes (**Figure 19**). HEK-293 cells are a human epithelial kidney cell line which exhibit enhanced attachment to poly-lysine coated surfaces as compared to tissue culture-treated surfaces. This is particularly important if the cells need to remain attached during subsequent washes (**Figure 20**). The appropriate 2D surface is determined by the cell type.

Three-dimensional growth substrates can support certain cellular behaviors that are not observed when cells are cultured on a planar two-dimensional surface, as exemplified by mammary epithelial50-54 and Caco-255-56 cells. *In vivo*, mammary epithelial cells form polarized acini. When tumorigenic human mammary carcinoma cells (T4-2) are cultured on a 3D substrate comprised of reconstituted basement membrane (Growth Factor Reduced Corning Matrigel® Matrix) they form large disorganized colonies, as shown with the T4-vector control in a study from Dr. Bissell's laboratory⁵¹ (**Figure 21**). Epidermal growth factor receptor (EGFR) had previously been shown to be elevated in T4-2 cells, and downregulation of this signaling pathway in T4-2 cells cultured in 3D Corning Matrigel Matrix is known to lead to phenotypic reversion to polarized acini. These cells exhibit polarized acinar architecture in the presence of the EGFR inhibitor AG1478 or when stably expressing dominant negative Rap1 (T4-DN-Rap1); reversion to a normal phenotype is shown by proper localization of α6-integrin (basal marker), β-catenin (basolateral marker) and GM130 (apical marker). These data show that three-dimensional Corning Matrigel Matrix culture conditions are conducive to studying signaling pathways involved in regulating mammary acinar architecture.

Another example of the effect of 3D growth substrates on cellular phenotypes is the use of Corning BioCoat Fibrillar Collagen Inserts in Caco-2 assays. Caco-2 cells are an epithelial cell line derived from a colorectal adenocarcinoma commonly used to measure compound permeability. The gold standard for modeling drug permeability across the intestinal epithelium *in vitro* is measuring permeability across differentiated Caco-2 cells, where the cells have been cultured for 21 days on cell culture inserts. Collagen BioCoat HTS Caco-2 Assay System and Corning BioCoat Intestinal Epithelium Differentiation Environment utilize Collagen BioCoat Fibrilliar Collagen Inserts and a specialized media to enhance the rate of Caco-2 differentation from 21 to 3 days (**Figures 22-23**), thereby reducing the time and labor required for the analysis of compound permeability.

The 2D and 3D cell culture systems available from Corning provide multiple options to researchers studying epithelial cells *in vitro*.

HEK-293 cells have enhanced attachment to Corning BioCoat Poly-D-Lysine Cultureware as compared to Corning Falcon Tissue Culture-treated Cultureware. An equal number of cells were plated on Corning BioCoat Poly-D-Lysine 384-well black/clear (right) and Falcon Tissue Culture-treated 384-well Black/Clear Plates (left) and grown under serum-free conditions. Before washing (top), there were a similar number of cells in the Corning BioCoat Poly-D-Lysine coated wells and the Falcon Tissue Culture-treated wells. After washing, using a Skatron Washer (Molecular Devices) (middle), the cells remained attached to the Corning BioCoat Poly-D-Lysine wells while few cells remained attached to the Falcon Tissue Culturetreated wells. Post-wash, the cells were visualized using Calcein AM (bottom).

Tools for Epithelial Cell Culture

Cat. No. Description Qty. Cell Culture Reagents

Cell Culture Tools

Membrane Insert Systems

For a complete product listing, see page 19.

FIGURE 21 • EFFECT OF RAP1 ACTIVITY ON T4-2 CELL POLARITY IN 3D GROWTH FACTOR REDUCED CORNING® MATRIGEL® MATRIX CULTURE

Corning Matrigel Matrix Growth Factor Reduced supports mammary acini formation *in vitro*. Malignant T4-2 cells were grown in three-dimensional culture on Corning Matrigel Matrix Growth Factor Reduced. Cells were stably transfected with control (T4-vector) or dominant negative-Rap1 (T4-DN-Rap1). Inhibition of EGFR with AG1478 was used as a positive control for reversion of T4-2 to normal mammary acinar architecture. Indirect immunofluorescence was used to analyze cell polarity markers for basal (α6-interin), basolateral (β-catenin) and apical (GM130) membrane domains. Bar, 5 µm. Images kindly provided by Dr. Masahiko Itoh and Dr. Mina Bissell, originally published in Cancer Research $67(10):4759-4766^{51}$. Reproduced with permission.

Caco-2 cells were cultured using the three-day Corning BioCoat HTS Caco-2 Assay System supplemented with MITO+ Serum Extender (A) or the traditional 21-day system (B). P-gp function was assessed by adding 10 nM ³H-labeled vinblastine in PBS to either the apical or basal side of the insert. Samples were withdrawn from the non-labeled side of the insert and counted by scintillation counting. To inhibit the P-gp with verapmil, 100 µM verapamil was added to the insert chambers.

FIGURE 22 • PERMEABILITY OF MANNITOL AND ANTIBIOTICS THROUGH CACO-2 MONOLAYERS

Barrier formation occurs three days postseeding in the Corning BioCoat™ Intestinal Epithelium Differentiation Environment and two to four weeks with conventional methods. Monolayers formed using either the Corning BioCoat Intestinal Epithelium Differentiation Environment or conventional methods are equally permeable for each of the three compounds tested.

Tumor Cells

Cancerous cells have altered cellular functions as compared to the normally functioning, non-malignant cells from which they are derived. Cell morphology and signaling pathway studies *in vitro* that incorporate the use of 3D culture systems can give insights into the effects of mis-regulated or mis-expressed proteins, as exemplified by human mammary carcinoma cells (T4-2)⁵¹ (Figure 20). The hallmark of metastatic cells is their ability to invade through the basement membrane and migrate to other parts of the body. Cell migration can be studied using either Falcon® Cell Culture Inserts or Corning® FluoroBlok™ Cell Culture Inserts for moderate to high-throughput screening (**Figure 24**). Cells must be able to both secrete proteases that break down the basement membrane as well as migrate in order to be invasive. Invasion through Corning Matrigel® Matrix-coated Cell Culture Inserts has become the gold standard for quantitative and qualitative measurement of the metastatic potential of a cell^{10, 57-63}. This matrix provides a true barrier to non-invasive cells while presenting the appropriate protein structure for penetration of invading cells.

Migration of Calcein AM (A) and DiIC₁₂(3) (B) labeled human fibrosarcoma cells (HT-1080) through Corning Falcon FluoroBlok 96-Multiwell Inserts, 8 µm pore size. DMEM with 5% FCS was used as a chemoattractant in the lower wells, while DMEM/0.1% BSA was added to the control wells. The plates were incubated for four hours at 37°C, after which fluorescence of cells which had migrated through the microporous membrane was measured on the Applied Biosystems CytoFluor® 4000 and PerkinElmer HTS 7000 Plus fluorescent plate readers using excitation/emission wavelengths of 485/530 nm for Calcein AM or 530/590 nm for DiIC₁₂(3). Values represent the mean of 8 wells \pm S.D. Migration from as few as 4,000 input cells can be detected.

Pseudo-colored image for illustrative purposes only.

 Cat No. **Description Qty**

Tools for Tumor Cell Culture

Membrane Insert Systems

For a complete product listing, see page 19.

DID YOU KNOW?

• Corning offers a full range of dishes and flasks. Please contact your sales representative for more information.

The Corning® BioCoat™ Matrigel® Invasion Chambers and Corning BioCoat Tumor Invasion Systems are optimized systems that utilize standardized coating procedures to ensure even coating of Corning Matrigel Matrix for reproducible results (**Figure 25**). The Corning BioCoat Tumor Invasion System provides a unique, quantitative platform that can be used to determine the effects of anti-metastatic compounds on invasive cell types (**Figure 26**). For *in vivo* studies, Corning Matrigel Matrix can be used to help support tumor cell engraftment in mice⁶⁴⁻⁶⁶. These tools allow researchers to dissect various areas of tumor biology, from analysis of signaling pathways *in vitro* to *in vivo* tumor formation.

Multiple lots of the Corning BioCoat 96-Multiwell Tumor Invasion System and Corning BioCoat 24-Multiwell Tumor Invasion System were assayed to show reproducibility with these systems. Multiple lots of Corning BioCoat 96-Multiwell Tumor Invasion System (A) and Corning BioCoat 24-Multiwell Tumor Invasion System (B) were assayed. Fluorescently labeled cells residing on the bottom of the insert membrane were measured post-invasion with either a Victor2™ plate reader (Corning BioCoat 96-Multiwell Tumor Invasion System) or a CytoFluor® plate reader (Corning BioCoat 24-Multiwell Tumor Invasion System). Mean percent invasion of NIH-3T3 and HT-1080 cells were compared. Cells were labeled post-invasion using Corning Calcein AM.

FIGURE 26 • INHIBITION OF PC3 MIGRATION AND INVASION BY DOXYCYCLINE

PC3 invasion is inhibited by doxycycline. PC3 cell invasion was measured using Corning BioCoat 24-Multiwell Tumor Invasion System, which is based on the fluorescence blocking Corning FluoroBlok™ PET microporous membrane, and migration was measured using Corning FluoroBlok 24-Multiwell Insert System. At the end of the assay, cells were stained with Corning Calcein AM.

Cell Culture Reagents

Extracellular Matrix Proteins

Cytokines and Media Additives

Corning Cell Recovery/Detachment Reagents

Cell Culture Tools

Corning® BioCoat™ Collagen I Cellware

Corning BioCoat Poly-D-Lysine Cellware

Corning® BioCoat™ Poly-L-Lysine Cellware

Corning BioCoat Laminin Cellware

Corning BioCoat Matrigel® Matrix – for Hepatocytes

Corning BioCoat Matrigel Matrix Plates for Embryonic Stem Cell Culture

Corning BioCoat Poly-D-Lysine/Laminin Cellware

Corning BioCoat Poly-L-Ornithine/Laminin Cellware

Falcon® Cultureware

Primaria™ Cultureware

Corning® Gentest™ Hepatocytes and Reagents

Transporter-Qualified Human CryoHepatocytes

Metabolism-Qualified Human CryoHepatocytes

Cell Environments

Membrane Insert Systems

For use with Falcon® Cell Culture Insert Companion Plates

Cell Environments (continued) Membrane Insert Systems (continued)

Membrane Insert Systems (continued)

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