Driving Cellular Communication – Effects of Culture Environments on Cell Growth and Differentiation

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BD Biosciences – Discovery Labware
Outline

• **Culture surfaces**
  – Synthetic
  – Biological – extracellular matrix (ECM)

• **Introduction to 2D vs 3D culture**

• **Cell type-specific examples**
  – Epithelial cells
  – Endothelial cells
  – Neurons
  – Human embryonic stem cells
How Surfaces Affect Cell Culture

• **Surface properties**
  – Surface charge, protein motifs
    • A misconception: more hydrophilic the better (Poly-lysine coated surface is more hydrophobic than Tissue Culture (TC)-treated surface)
  – 2D vs 3D structure

• **Effect of surface is dependent on the culture environment**
  – Cell type
  – Media composition
  – Incubation time, etc.
Culture Surfaces

- **Vertebrate cells have negative surface charge**
  - Cells spread using the ECM they lay down as well as serum or media derived attachment factors

- **Synthetic surfaces**
  - Negative: glass, TC-treated polystyrene
  - Positive and negative: BD Primaria™ Cultureware
  - Positive: poly-lysine

- **ECM components (2D or 3D)**
  - Fibronectin is a key attachment factor derived from serum
  - BD BioCoat™ Cultureware (ex., Collagen I, Collagen IV or Fibronectin)
  - Reconstituted basement membrane (ex., BD Matrigel™ Matrix)

- **Microporous membranes (cell culture inserts)**
Synthetic Surfaces: Surface Chemistry

• Non-treated polystyrene
  – Ex. BD Falcon Non-treated Surface
Synthetic Surfaces: Surface Chemistry

• **TC-treatment**
  – Rendered hydrophilic by a process that adds a variety of negatively charged functional groups to the surface
  – Ex. BD Falcon™ TC-treated Surface
    • Vacuum-gas plasma treatment – oxygen
    • Specific conditions of pressure and temperature

![Chemical structure](image)
Synthetic Surfaces: Surface Chemistry

• **BD Primaria™ Surface**
  – Vacuum-gas plasma treatment – oxygen + ammonia
  – Integrates **amine and amide functional groups** with the traditional hydroxyl/carboxyl TC-surface chemistry
Synthetic Surfaces: Cell Growth on Polystyrene Substrates

Chinese hamster ovary (CHO) cells, 72 hours after seeding, 10% serum
Synthetic Surfaces: Cell Growth on Polystyrene Substrates

LNCaP (human carcinoma) cell, 24 hours after seeding, 10% serum

Non-treated Polystyrene

BD Falcon™ TC

BD Primaria

MTS assay

OD\textsubscript{490}

Non-treated Polystyrene

BD Falcon TC

BD Primaria
Synthetic Surfaces: Cell Growth on Polystyrene Substrates

3T3 (mouse fibroblast) cells, 24 hours after seeding, serum-free media

Non-treated Polystyrene

BD Falcon™ TC

BD Primaria

MTS assay

OD$_{490}$

Non-treated Polystyrene  BD Falcon TC  BD Primaria
Synthetic vs. Biological Surfaces

- **Synthetic**
  - Covalent modification of polystyrene
    - TC-treated
    - BD Primaria™ Cultureware
  - Coated polystyrene surface
    - Poly-lysine

- **Biological**
  - Coated polystyrene surface
    - ECM protein(s)
    - Thin layer (2D)
    - Gel (3D)
Biological Surfaces: ECM Proteins

- ECM molecules interact with cell surface receptors (e.g., regulation of integrin signaling by fibronectin:integrin interactions)
- ECM appears to function in the storage and presentation of growth factors
- ECM components
  - ex., Fibronectin, Laminin, Collagen
Biological Surfaces: ECM Proteins

• **Fibronectin (FN)**
  – Large dimeric protein *(multiple isoforms)*
  – Contributes to matrix organization
  – Promotes cell adhesion via interaction between FN ‘**RGD motif**’ and integrin receptors
  – Promotes cell differentiation and functionality (e.g., cell migration, integrin signaling, gene expression)
• **Laminin (LM)**
  – Large heterotrimeric proteins
  – Primarily found in basal lamina
  – Major structural component of basal lamina
  – Promotes cell adhesion via integrin and non-integrin receptors
  – Promotes cell differentiation and functionality (e.g., neurite outgrowth, receptor signaling, gene expression)
Biological Surfaces: ECM Proteins

- **Collagen**
  - Most ubiquitous ECM molecules
  - Fibrous proteins that provide structure and resiliency to tissues
  - Major component of skin
Biological Surfaces: Reconstituted Basement Membrane

• **BD Matrigel™ Matrix**
  - Purified preparation from EHS mouse tumors
  - Composition
    • Laminin ~60%
    • Collagen IV ~30%
    • Entactin ~8%
    • Heparan sulfate proteoglycan (perlecan)
    • Growth factors (e.g., PDGF, EGF, TGF-β)
    • Matrix metalloproteinases
2D vs. 3D Cell Culture: Cell Differentiation and Function

- Biological composition of the culture environment
  - Cell type(s)
  - ECMs
  - Growth factors
- Molecular interactions and cell adhesion
  - Cell:cell, cell:ECM, cell:growth factor
  - ECM:ECM, ECM:growth factor
- Mechanical strength and structural properties
  - Degree of rigidity
  - 3D architecture
- Size scale
  - Pore or fiber size relative to cell size (microfibers vs. nanofibers)
## 2D vs. 3D Cell Culture

<table>
<thead>
<tr>
<th></th>
<th>2D</th>
<th>3D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Substrate</strong></td>
<td>Rigid; inert</td>
<td>Mimics natural tissue environment</td>
</tr>
<tr>
<td><strong>Architecture</strong></td>
<td>Not physiological; cells partially interact</td>
<td>‘Physiological’; promotes close interactions between cells, ECMs, growth factors</td>
</tr>
<tr>
<td><strong>Cell Encapsulation</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Growth Factor Diffusion</strong></td>
<td>Rapid</td>
<td>Slow; chemical and biological gradients regulate signaling, cell-cell communication</td>
</tr>
</tbody>
</table>
2D vs. 3D Cell Culture: Surfaces

- **2D culture surfaces**
  - Synthetic
    - Ex. TC, BD Primaria™, Poly-lysine
  - Biological
    - Ex. Fibronectin, Collagen, Laminin

- **3D culture surfaces**
  - Synthetic
    - Ex. BD™ PuraMatrix™ Hydrogel
  - Biological
    - Ex. BD Matrigel™ Matrix, Collagen, Laminin/Entactin
Factors in Surface Selection

- Assay
  - Undifferentiated cells
  - Differentiation
- Media
- Fast attachment
- Strong attachment
- Cell type

MCF-7 (breast cancer cell line) labeled with Hoechst (blue), Mitotracker green (green) and wheat germ agglutinin (red). BD Pathway™ 855 High-Content Bioimager.
Factors in Surface Choice: Media

- **Serum containing media**
  - Culture surface may be coated with serum proteins – partially or entirely screen out underlying surface properties
  - Surface properties may affect cell attachment through the selective binding of proteins

- **Reduced serum or serum-free media**
  - Underlying surface properties may become more important
Factors in Surface Choice: Media

MRC5 (human fibroblast) cells

Non-treated Polystyrene

5% serum

1% serum

BD Falcon™ TC-treated
Factors in Surface Choice: Rapid Attachment

BHK21 (hamster fibroblast) cells, 1 hour after seeding
Factors in Surface Selection: Strength of Attachment

Transfected HEK-293 (human kidney epithelial) cells, 24 hours in serum-free media

Samples were washed using a Skatron Washer (Molecular Devices)
Factors in Surface Selection: Cell Type

- Epithelial cells
- Endothelial cells
- Neurons
- Human embryonic stem cells
Epithelial Cells

- Line all cavities and free surfaces of the body
- Tightly bound into sheets ‘epithelia’
- Epithelia are barriers to water, solutes, cells
- ECM that underlies epithelia: basal lamina
- Examples
  - Liver (hepatocytes)
  - Skin (e.g. keratinocytes)
  - Gut, Lung
  - Exocrine glands (e.g., mammary, sweat)
Epithelial Cells: Hepatocytes

- **Applications**
  - Drug metabolism studies, toxicity assays
  - Liver regeneration, tissue engineering
  - Liver-specific gene expression and signaling
Epithelial Cells: Hepatocytes

Cryopreserved human hepatocytes, thawed and plated for 4 hours

Surface treatment required for hepatocyte attachment.
Epithelial Cells: Hepatocytes

Primary Rat Hepatocytes

Col I (2D thin coat)  Col I (3D gel)  BD Matrigel™ Matrix

Hepatocyte morphology and function altered by surface treatment.
EcoPack™ 2-293 Cells (derived from HEK-293)

Integrin $\alpha_v$ expression is affected by the growth surface.
Epithelial Cells: Caco-2

Caco-2 cells, thawed and cultured for 24 hours

TC-treated

BD BioCoat™ Collagen I
## Epithelial Cells: Caco-2

### Digoxin Permeability Comparison

<table>
<thead>
<tr>
<th>Caco-2 Line</th>
<th>Pore Size</th>
<th>Papp A-B</th>
<th>Papp B-A</th>
<th>Papp Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% FBS</td>
<td>1 uM</td>
<td>mean:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.9</td>
<td>9.7</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD:</td>
<td>0.8</td>
<td>1.8</td>
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<tr>
<td></td>
<td></td>
<td>%CV:</td>
<td>27</td>
<td>19</td>
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<tr>
<td></td>
<td></td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% FBS</td>
<td>1 uM</td>
<td>mean:</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1.3</td>
<td>17.3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD:</td>
<td>0.24</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%CV:</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=8)</td>
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<td></td>
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</tbody>
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Initial growth conditions affect cell Caco-2 function.
Endothelial Cells

- Line closed internal body cavities (e.g. blood vessels)
- Angiogenesis (e.g., neovascularization during wound healing and tumorigenesis)
  - Formation (sprouting) of capillaries from existing small blood vessels
  - Distinct from vasculogenesis, which is de novo synthesis of vessels from angioblasts (endothelial progenitor cells)
  - Required for tumor growth and survival
- Promote blood cell adhesion during inflammatory response
Endothelial Cells: HUVEC

Human Umbilical Vein Endothelial Cells (HUVEC)

TC-treated

BD BioCoat™ Collagen I
Endothelial Cells: FBHE cells

Fetal Bovine Heart Endothelial (FBHE) Cells

TC-treated

BD BioCoat™ Collagen I
Endothelial Cells: Differentiation

Human Microvascular Endothelial Cells

Collagen I

BD Matrigel™ Matrix
Endothelial Cells: BD BioCoat™ Angiogenesis Systems

- **Endothelial Cell Migration**
  - 24- or 96-Multiwell BD FluoroBlok™ Insert (3 μm pore size)
  - Coated with Human Fibronectin

- **Endothelial Cell Invasion**
  - 24-Multiwell BD FluoroBlok Insert (3 μm pore size)
  - Coated with BD Matrigel™ Matrix

- **Endothelial Cell Tube Formation**
  - Comprised of a BD Falcon™ 96-well black/clear plate coated with BD Matrigel Matrix (non-insert system)

- **BD Human Umbilical Vein Endothelial Cells** (BD™ HUVEC-2)
  - Pre-qualified for VEGF responsiveness and for use with migration assay; also suitable for use with invasion and tube formation assays
Neurons

• Primary Neurons
  – Central nervous system/brain
  – Peripheral neurons (e.g., sensory, motor)

• Neurons
  – Axons conduct and transmit signals
  – Dendrites receive signals
  – Neuronal communication mediated by neurotransmitters (e.g., glutamate, dopamine, serotonin, GABA)
Neurons: Rat Cortical Neurons

Glutamate receptor activity on BD BioCoat™ Laminin/Fibronectin Cultureware.
Neuronal Cells: PC-12

PC-12 (rat pheochromocytoma) cells, 24 hours after seeding, 10% serum

MTS assay

- OD$_{490}$

<table>
<thead>
<tr>
<th>Condition</th>
<th>OD$_{490}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Falcon™ TC</td>
<td>0.2</td>
</tr>
<tr>
<td>BD BioCoat™ Poly-D-Lysine</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Neurons: PC-12

PC-12 cells, grown on Collagen I

Control

200 ng/ml NGF

Neurite outgrowth after 10-day treatment with NGF.
Visualized with anti-β-tubulin and Hoechst using BD Pathway™ Bioimager.
Human Embryonic Stem Cells

• Generated from the inner cell mass of blastocyst
• Pluripotent cells
• Maintaining pluripotency requires specific culture conditions
  – BD Matrigel™ Matrix
  – BD Laminin/Entactin
Human Embryonic Stem Cells: Surfaces

MEF-CM

mTeSR™ 1

BD™ Laminin/Entactin Complex High Concentration

BD Matrigel™ hESC-qualified Matrix
Human Embryonic Stem Cells: Surfaces

Pluripotency marker, OCT-4, expression in H9 cells.
Summary

• Cell / surface interactions are very complex
• Currently, our main approach is empirical
• Ultimate goal – predict the surface properties needed for certain cell types and culture conditions
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