Dynamics of Neurons in Culture

Motile machinery underlying development and growth in 2D and 3D matrices.
Neuronal Tissue Culture

- A reductionist experimental approach to mimic aspects of neuronal development, maintenance (plasticity/regeneration), and degeneration
- Dissociated cells, explants, tissue slices, 3D
- Basal medium, additives/supplements, serum, antibiotics
Hierarchy of Morphological Complexity
Neuronal Plasticity

Learning

Stress

Alcohol

Sugar

Aging

Drugs

young

old

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Connectivity and Density
Neuronal Tissue in Experimental Systems
Neuronal Tissue Culture

• Loss of complexity, morphological detail, cell-cell contact/density

• Most neuronal tissue culture relies embryonic rodents (genetically homogenous cells)

• Many unusual conditions including O₂ pressure, adhesion substrates, glucose, antibiotics, intercellular space.....

• Tissue culture utilized room air (21% O₂ or 160 mHg pO₂) in contrast to pO₂ in blood (80 mmHg) or brain tissue (25-50 mmHg)

• Glucose concentration in blood (3-7 mM), in brain (1-3 µM) compared to 25-50 mM in DMEM or Neurobasal

• Substrates – plastic, poly-D-lysine, laminin, fibronectin, collagen, ECM matrix.....
Neuronal Dynamics

• Morphology of neurons exhibits persistent dynamics
• Essential for development, maintenance, and regeneration of neuronal connectivity
• Profoundly influence by adhesion contacts (substrates)
• Dynamic network of actin filaments, myosin family members, and tubulin filaments
• Force generation through adhesion contacts
Growth Cones Motility and Actin Dynamics

- tips of elongating neuronal processes
- highly motile
- numerous sensory capacities
- autonomous
- lamellipodia and filopodia
- actin-based motility
Growth Cone Motility and Signal Integration

Attraction

Repulsion

Δ Actin Cytoskeleton

Δ Behavior

Rho GTPases (Rac1)

- TNFα, IL-1β
- Semaphorins
- Netrins
- MAG
- NOGO
- Ephrins
- CSPG
- Abeta
- HIV gp120

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Extrinsic Signals alter Neuronal Dynamics
Redox-dependent Impairment of Neuronal Motility by Cytokines

Kuhn, BMRI 2014
Growth Cone Motility
Rac1 and Oxygen Radicals

TNFα, IL-1β → Rac1A → ROS → Actin Reorganization → Growth Cone Motility

Graphs showing:
- Rac1A Activity
- Longest Neurite
- Relative amounts of actin filaments per growth cone

- Control
- V12Rac1
- N17Rac1

% neurons with neurite length > X

X = neurite length (µm)
Extrinsic Signals and Polarized Neuronal Morphology

Extrinsic Signal (acute or chronic)
Isolation of Growth Cone Responses

IN VITRO

IN VIVO

Signal

Immobilized Signal

Laser Tweezers

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• 78±4% responding growth cones
• lamellipodia induction 4.8±0.8 min following initial bead contact
• duration of lamellipodial activity = 5.8±0.6 min
Local Contacts ⇒ Transient Signals

A, B: Growth cone - Laminin contact

C, D: Neurite - Laminin contact

Cognitive decline is a progressive loss of the integrity and plasticity of neuronal connectivity

= Neurodegeneration

Hallmark:
Inflammatory and Oxidative Stress
Lipid Rafts: Origins of Neurodegeneration?

HIVgp120

$\beta$-amyloid ($\alpha$P)

TNF$\alpha$

PrP$\text{Sc}$

PrPC

Lipid raft domain

Rac1

NOX

Cofilin dimers

O$_2$

Superoxide

Rods

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3D Culture Novelties

• Provide cells with a more physiological environment
• Less rigid 3D scaffold with diverse adhesion option
• Enhanced proximity to neighboring cells
• Greatly reduced intercellular space

Figure 11.1 Electron micrographs of neuronal cultures in 2D (left) and 3D (right). Neurons in 2D have a flattened morphology, while neurons cultured in a 3D matrix of Matrigel present a rounded morphology with matrix interactions possible in all spatial dimensions. (The authors thank Dr. Robert P. Apkarian, the director of the Integrated Microscopy and Microanalytical Facility at Emory University, for his assistance in electron microscopy.)
<table>
<thead>
<tr>
<th>Cellular characteristics</th>
<th>2D</th>
<th>3D</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Sheet-like flat and stretched cells in monolayer</td>
<td>Natural shape in spheroid/aggregate structures</td>
<td>20,24,50</td>
</tr>
<tr>
<td>Proliferation</td>
<td>Often proliferate at a faster rate than <em>in vivo</em></td>
<td>May proliferate at a faster/slower rate compared to 2D-cultured cells depending on cell type and/or type of 3D model system</td>
<td>17,51</td>
</tr>
<tr>
<td>Exposure to medium/drugs</td>
<td>Cells in monolayer are equally exposed to nutrients/growth factors/drugs that are distributed in growth medium</td>
<td>Nutrients and growth factors or drugs may not be able to fully penetrate the spheroid, reaching cells near the core</td>
<td>24,52</td>
</tr>
<tr>
<td>Stage of cell cycle</td>
<td>More cells are likely to be in the same stage of cell cycle due to being equally exposed to medium</td>
<td>Spheroids contain proliferating, quiescent, hypoxic and necrotic cells</td>
<td>18,24,53</td>
</tr>
<tr>
<td>Gene/protein expression</td>
<td>Often display differential gene and protein expression levels compared to <em>in vivo</em> models</td>
<td>Cells often exhibit gene/protein expression profiles more similar to those <em>in vivo</em> tissue origins</td>
<td>17,40,54</td>
</tr>
<tr>
<td>Drug sensitivity</td>
<td>Cells often succumb to treatment and drugs appear to be very effective</td>
<td>Cells are often more resistant to treatment compared to those in 2D culture system, often being better predictors of <em>in vivo</em> drug responses</td>
<td>17,33</td>
</tr>
</tbody>
</table>

2D, two-dimensional; 3D, three-dimensional
2D Cultures

• Highly rigid adhesion
• Altered gene expression due non-physiological adhesion
• Low density cell cell interactions
• Rapid dilution of factors
• Easy observation and manipulation
• Too much surface exposure
• Vast literature

3D Cultures

• Less rigid “physiological” adhesion microenvironment
• High density, multiple cell cell interactions
• Slow diffusion, real gradients of signals
• Neuronal process grow 3 D
• More in vivo like
• Potential for nutrient deprivation
• Less access to manipulation and observations
Lessons Learned

• 3D neurons response different to pharmacology (AD γ-secretase/BACE inhibitors)
• Neurite outgrowth in 3D is enhanced
• Synaptic density in 3D is vastly increased
• Ion channel physiology is enhanced in 3D neurons
• Gene expression (possibly epigenetics) is vastly different in 3D cultures

Cells in 2D conditions are vastly different from cells in 3D conditions thus exhibit distinct behaviors and response to extrinsic and intrinsic signals
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