Modeling Ischemic Stroke In Vitro: Status Quo and Future Perspectives

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Stroke is a devastating disease accounting for 5.5 million deaths annually worldwide. Despite significant preclinical and clinical investigations, with >1026 candidate neuroprotective stroke drugs investigated and ≈200 clinical trials, no effective therapy other than tissue-type plasminogen activator has been approved. The reasons for these failures are numerous. Recent Stroke Treatment and Academic Roundtable guidelines focusing on the appropriate use of animal models and clinical trial design will undoubtedly improve the odds of identifying effective therapeutics. However, improvements will need to be made across all levels of the drug discovery pipeline if new therapies are to be effectively identified and developed. This review will cover current methods used for modeling ischemic stroke in vitro, along with some of the insights that have been gained and the technological developments that may allow for the production of more effective and relevant models for research in stroke.

In Vivo Versus In Vitro

In vivo models have enabled a great insight into the pathophysiology of human disease and have been critical in our understanding of stroke. Rodents are often the chosen species, because of availability of genetically altered strains. However, ≈70 million years of evolution, separate humans from rodents and just a 10% difference in genome implies that ≈3000 genes differ. Aside from macrostructural discrepancies, many cellular and molecular differences exist. For example, the expression levels of transporters and pumps that contribute to the blood–brain barrier (BBB), and the functional diversity and abundance of astrocytes distinguish the human BBB from the rodent. Duration of excitotoxicity has also been demonstrated to differ significantly between humans and mice.

Considering the large inflammatory component of stroke pathology, it is also important to note differences in immune biology between species. Many important immune signaling molecules (interleukin-8, chemokine (C-X-C motif) ligand 7, chemokine (C-C motif) ligand [CCL] 18, monocyte chemoattractant protein-4 and CCL24/CCL26) are expressed in humans but not in mice, whereas CCL6, CCL9, and monocyte chemoattractant protein-5 are present in mice but not in humans. As such, the possibility of therapeutic targets being present in humans, but not in rodents, or vice versa, should not be overlooked.

Certain non-human primates provide a more anatomically and genetically relevant model species; however, even primates’ genes with homology have been shown to have evolved with different biochemistry and function. This is exemplified by the recent clinical trial of TGN1412 that almost resulted in the deaths of 6 healthy volunteers. Differences in binding characteristics have now been found to account for the severe inflammatory response seen in humans, but not in rhesus monkeys. Since then in vitro testing of TGN1412 in appropriate human cell, in vitro models have replicated the dramatic cytokine release that evaded previous preclinical trials. Thus, human in vitro systems may provide highly valuable information as to a drug’s safety while also allowing for cost effective high-throughput (HT) screening assays. However, of 100000 studies on stroke investigated in 2011, only 5 were performed in human in vitro systems. With the advent of new cellular technologies and genetic and molecular manipulation, there is a great opportunity for the development of in vitro systems to model stroke and improve the drug discovery pipeline.

Modeling Stroke In Vitro: Causing the Damage

Chemical and Enzymatic Methods

Ischemic conditions can either be modeled by removal of oxygen and glucose or via chemical or enzymatic inhibition of metabolism. Kurian and Pemaih have previously compared many chemical and enzymatic methods against oxygen and glucose deprivation (OGD) with reperfusion in a renal epithelial cell model, to our knowledge no such comparison has been made for stroke models. In this study, they compared chemical methods using rotenone, antimycin, and sodium azide to inhibit the electron transport chain. The enzymatic methods used were the glucose oxidase/catalase system consisting of and catalase and 2-deoxyglucose (an isomer of α-glucose, which cannot be metabolized). These methods were compared with OGD for 1 hour followed by 3-hour reperfusion. Antimycin-mediated cell injury was found to be the most
reproducible, whereas OGD required a much longer time to produce cell injury.

Although chemical and enzymatic methods may be suited to HT, because of ease of application and rapidity of response, this is at the expense of relevance (for instance, chemical hypoxia results in more free radical generation than anoxia).

**Oxygen and Glucose Deprivation**

Ischemia-like conditions can be induced by replacing the normal O$_2$/CO$_2$ equilibrated medium with N$_2$/CO$_2$ equilibrated medium and maintaining cells in a hypoxic chamber. Although oxygen deprivation is usually combined with glucose deprivation, many in vitro studies have demonstrated that hypoxia alone causes dramatic alterations in endothelial cell (EC) actin cytoskeleton and tight junction protein localization in BBB models. However, the physiological relevance of anoxia in the absence of glucose depletion is most relevant to conditions where blood flow is maintained, such as carbon monoxide poisoning, thus, in the modeling of stroke, OGD is most relevant. In murine primary cortical cultures, the rate of neuronal cell loss in anoxia is dependent on the concentration of glucose in the media. Half maximal neuronal loss occurs by 4 to 8 hours with 2 mmol/L glucose and in >24 hours with 20 mmol/L glucose (a concentration typical of most classical media). GD alone is rarely used to model stroke and >4 hours does not significantly influence survival of rat cortical neurons in culture. However, in organotypic mouse hippocampus cultures, GD alone for 2.5 hours resulted in significant CA-1 cellular damage. Such discrepancies may be explained by the relative sensitivity of CA-1 neurons and highlight the importance of using heterogeneous cell populations.

Combining OGD causes primary neurons to undergo acute cell body swelling within <60 minutes followed by apoptotic and excitotoxic necrotic cell death. In OGD with reperfusion experiments, neuronal degeneration occurs over several hours, despite return to standard culture conditions, as is consistent with observations of in vivo ischemia-reperfusion injury. OGD in primary mouse cortical neurons is also associated with a large increase in extracellular glutamate concentration consistent with excitotoxic effects in vivo.

**Excitotoxicity**

In vitro models also allow for the study of excitotoxicity in isolation of ischemic damage, through application of glutamate receptor agonists such as N-methyl-D-aspartate or glutamate. The use of specific agonists allows investigation of the involvement of glutamate receptor subtypes in excitotoxic events.

**Cellular Platforms Used in Stroke Models**

An overview of the cellular platforms used in stroke research is given in the Table.

**Brain Slices**

The brain slice method uses a thin slice of brain tissue (usually 400 μm), allowing for an in-depth probing of neuronal circuitry. The slice is perfused with artificial cerebral spinal fluid, allowing rodent slices to be maintained for ≤12 hours. Removing glucose and replacing oxygen with nitrogen in this solution provides global OGD, causing neuronal and astrocyte depolarization within 10 minutes in rat cortical slices. The benefit of preserving architecture is that different vulnerabilities of neuronal cell types may be assessed. In addition, neuronal effects of ischemia can be separated from cerebrovascular influences. Richard et al have built on this model to provide focal OGD achieved by perfusing OGD artificial cerebral spinal fluid over a brain slice in a focal stream, whereas the remainder of the slice is perfused with normal artificial cerebral spinal fluid. Using this model in rodent brain slices, Richard et al have demonstrated rapid neuronal depolarization in the anoxic core, followed by progressive depolarization in the penumbra region.

A key benefit of in vitro systems is the opportunity to work with human cells, as such Werth et al used the brain slice method in human cortical slices to provide the first direct evidence of glutamate receptor involvement in ischemic injury in the human brain. However, such investigations are low throughput, and availability of human samples is extremely limited. These samples are often obtained from neurosurgery of young epileptic patients, which may not represent the chemical and molecular physiology of the patient with typical stroke.

Although it has been recognized that human brain slices might provide a crucial intermediate assay to forecast translational success, it should be recognized that preparation of the slice is associated with tissue trauma and ischemia, and as such, basal level readings obtained represent a post-traumatic state.

**Organotypic Cell Culture**

Existing in the realm between brain slice and primary cell culture, these ex vivo cultures are obtained from different anatomical regions of the brain, usually taken from neonatal animals, and allowed to mature in vitro. This method maintains structural organization; however, many cultures experience synaptic rearrangement because of lack of extrinsic afferent and efferent signals in vitro. These cultures have increasingly been used to investigate neuronal cell death, myelination, synapse plasticity, and potential stroke therapies in OGD models; however, they use neonatal rodent tissue, which may be of questionable relevance to the adult-aging human.

**Cell Culture**

Cell cultures, while lacking the physiological architecture and networks that exist in vivo, are compatible with HT and allow for dissection of the contributions of individual cell types to pathological processes.

**Primary Cells**

The particular benefit of primary cells is evident in brain EC (BEC) primary cultures, where transendothelial electrical resistance (TEER) is comparatively high when compared with cell lines. Primary glial cell cultures are some of the most commonly used in vitro model for neurobiological studies and the molecular properties and differentiation of these cells in culture reflect their in vivo phenotype well. Primary cell isolation and purification is time consuming, yields can...
be limited, and possible contamination with other cell types raises questions of reproducibility.

Primary cells may also lose phenotypic identity with increased passage number, thus application to HT is limited. Furthermore, neurological research is particularly limited, because of the fact that once terminally differentiated into mature neurons, these primary cells can no longer replicate. On the contrary, human blood samples are readily available as such isolated primary leukocytes, and platelets may be used within a stroke model, to investigate relevant thrombotic and inflammatory responses.

**Cell Lines: Immortal Cancers With Mistaken Identities or Useful Tools?**

The main advantage of immortalized cell lines is the ready supply of human cells suitable for use in HT screening assays. Immortalized human microglia cells have been produced (eg, HMO6), established from primary human embryonic microglia and display a wide variety of inflammatory responses typical of the primary cell. The human teratoma-derived NT2 cell line is also a promising investigational tool from which neuronal cells, astrocytes, and oligodendrocytes can be derived. However, it should be noted that these cells contain oncogenes that distinguish them from endogenous cells by increased proliferation, cell adhesion, and variance of morphologies. The origin of the cell line should also be considered, for instance, SH-SY5Y is often used with OGD to model stroke; however, these adrenergic and dopaminergic cells are derived from cells of a bone marrow biopsy taken from a 4-year-old woman with neuroblastoma. Differences between primary and immortalized cells are also notable in BEC cell lines where BBB-specific adhesion molecules, enzymes, and transporters are expressed at much lower levels. Stem Cells

Embryonic stem cells in principle allow for unlimited quantities of every cell type in vitro, and numerous techniques have been established for the generation of lineage-restricted neural progenitor cells followed by their specific differentiation into neurons, astrocytes, or oligodendrocytes. Ethical issues limit the use of human embryonic stem cells; however, reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) provides an attractive alternative.

Since the first reprogramming of somatic cells to iPSCs by Yamanaka et al new strategies have been developed to induce iPSCs omitting oncogenic factors, using non-viral methods and removing transgenic sequences using recombination methods, such as Cre-loxP or PiggyBac transposition. Furthermore, protocols for iPSC cell differentiation to neurons are improving to give ≥100% purity of neurons. iPSCs have been used to model several diseases for drug screening, and since 2008 have also been used in modeling neuronal disease. The particular benefit of these cells is in producing patient-specific cells that can aid in modeling neurological disorders with significant genetic contributions, such as multiple sclerosis. Although stroke is a multifactorial disease, Mendelian stroke syndromes do exist, such as Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy. The use of iPSCs from such patients may aid a further understanding of stroke pathophysiology.

**Modeling the BBB in Stroke**

BBB compromise is an important pathological process in stroke causing further disruption of brain homeostasis. A wide variety of in vitro BBB models have been described, to assess both the effects of ischemic damage and potential therapeutic interventions to combat BBB breakdown, a thorough review is given by Gumbleton and Audus. Such models generally consist of a 2-layer or transwell cell culture system where permeability to molecules such as sucrose or TEER can be measured across the monolayer (Figure 1A). Commercially available 3-dimensional (3D) models allowing coculture have also been more recently developed (Figure 1B).

### Table. Comparison of Suitability of Cellular Platforms for In Vitro Neurological Research

<table>
<thead>
<tr>
<th>Cellular Platform</th>
<th>HT Ready</th>
<th>Availability of Human Cells</th>
<th>Physiological Relevance</th>
<th>Structural Complexity Modeled</th>
<th>Possible Application to Stroke Research</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain slice</td>
<td>No</td>
<td>Extremely limited</td>
<td>High</td>
<td>To a high degree</td>
<td>OGD, chemical ischemia, excitotoxicity</td>
<td>Tissue damage during preparation</td>
</tr>
<tr>
<td>Organotypic cell culture</td>
<td>No</td>
<td>Limited</td>
<td>High</td>
<td>To some degree</td>
<td>OGD, chemical ischemia, excitotoxicity</td>
<td>Tissue damage during preparation</td>
</tr>
<tr>
<td>Primary cells</td>
<td>No</td>
<td>Limited</td>
<td>High</td>
<td>Artificial arrangement required</td>
<td>OGD, chemical ischemia, excitotoxicity BBB models, thrombosis and leukocyte responses</td>
<td>Limited proliferative capacity</td>
</tr>
<tr>
<td>Cell lines</td>
<td>Yes</td>
<td>Unlimited</td>
<td>Low</td>
<td>Artificial arrangement required</td>
<td>OGD, chemical ischemia, excitotoxicity BBB models</td>
<td>Oncogenes present and high passage may limit physiological relevance</td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td>Partial</td>
<td>Extremely limited</td>
<td>Potentially high</td>
<td>Artificial arrangement required</td>
<td>OGD, chemical ischemia, excitotoxicity, BBB models</td>
<td>Limited use due to ethical issues</td>
</tr>
<tr>
<td>iPSCs</td>
<td>Yes</td>
<td>Potentially unlimited</td>
<td>Potentially high</td>
<td>Artificial arrangement required</td>
<td>OGD, chemical ischemia, excitotoxicity, BBB models</td>
<td>Single donor models of the relevant at-risk populations are possible</td>
</tr>
</tbody>
</table>

BBB indicates blood–brain barrier; HT, high-throughput; iPSCs, induced pluripotent stem cells; and OGD, oxygen glucose deprivation.
Build on BBB Models: The Neurovascular Unit

BBB models using BECs alone have low TEER and improper transporter and junctional protein localization when compared with those found in vivo. However, it is now recognized that the BBB is supported by multiple heterotypic cell interactions as a part of a neurovascular unit, involving astrocytes, pericytes, microglia, neurons, and also extracellular matrix components.

Astrocytes

Astrocytes, the most abundant glial cells in the brain, whose end-feet cover ≥99% of the vascular surface and contribute to the unique properties of BECs and thus BBB integrity. In agreement with this hypothesis, coculturing astrocytes with BECs significantly increases TEER and enhances the expression of transporters, BBB enzymes, and tight junctions. Astrocytes also actively participate in stroke pathology, and exposing BECs to media from primary astrocytes which have experienced hypoxia, stimulates inflammatory genes in BECs. Astrocytes have also been shown to influence immune cell recruitment to BECs. Such coculture methods may be used to investigate astrocyte influence on stroke-induced BBB breakdown.

Pericytes

Pericyte numbers in the brain correlate with BBB tightness, and their loss contributes to BBB breakdown in vivo. In vitro pericytes have also been shown to promote BBB characteristics in BECs. Thus, in vitro models that include pericytes may provide an insight as to whether these cells might present as a potential therapeutic target for the treatment of stroke and other neurovascular diseases.

Microglia

The role of microglia in regulating BBB remains controversial. Microglial activation promotes the restoration of a disrupted BBB, yet microglia-released tumor necrosis factor-α promotes BBB compromise. Such apparently contradictory results are likely because of the complex microglial phenotype.

Figure 1. Currently used blood–brain barrier (BBB) models. A, Diagrams demonstrating the varying complexities of transwell BBB models showing direct and indirect coculture methods; BECs may be cultured alone on a porous membrane transwell across which electrical resistance may be measured to assess BBB integrity. Astrocytes may be cocultured in the basolateral side of the well to enhance BBB properties, whereas in more complex models, pericytes or microglia may be included and cultured on the membrane. B, The commercially available Flocel Inc. DIV-BBB3D system uses a series of hollow fibers to allow for 3-dimensional coculture of BECs and astrocytes, while also allowing luminal flow to investigate leukocyte transmigration. Reprinted from Cucullo et al with permission of the publisher. Copyright ©2011, Macmillan Publishers Ltd. ECS indicates extraluminal space.
and activation state. Kaushal and Schlichter recently used a novel in vitro model of penumbral damage to help delineate the microglial involvement in stroke. In this model, neuron and astrocyte cocultures undergo OGD to simulate the stroke core. Soluble factors from the stressed cocultures are then used to activate the microglia. These microglia are then incubated with naïve neuron/astrocyte cocultures to reveal how microglia may damage healthy tissue. Such studies allow for insights into the complex role of microglia in BBB regulation.

Neurons
Considering that salvaging neuronal function is the aim of many prospective stroke treatments, incorporation of neurons in models of stroke is desirable. Although simplified models of ischemic damage or excitotoxicity regularly use neuronal cell cultures, their inclusion in models of the BBB is rare. Neurons have, however, been shown to promote BBB properties in BEC cultures, suggesting that inclusion of neuronal cultures in BBB models may provide useful mechanistic insights into ischemia-induced BBB breakdown.

Extracellular Matrix
The importance of extracellular matrix is exemplified by Tilling et al using porcine BECs. These experiments with various combinations of matrix coatings demonstrate significant effects on BEC permeability.

Beyond the Brain: Recruitment of Circulating Cells
Although most in vitro stroke models focus on brain parenchymal cells and BBB, it is important to recognize the significant contribution of circulating immune cells. There are many systems in which leukocyte recruitment can be assessed: from static transmigration assays through membranes, to static or flow-based leukocyte–EC interaction assays. However, few have used these assays to investigate stroke-specific leukocyte recruitment.

Studies by Inglis et al have shown that neutrophil migration across BECs in response to a chemotactic gradient decreases TEER. This is consistent with neutrophil migration in vivo causing vasogenic cerebral edema and BBB breakdown after stroke. OGD has also been shown to upregulate BEC adhesion molecules, chemotactic factors, and neutrophil binding under static conditions. Currently, we are establishing leukocyte–EC recruitment flow assays with OGD to dissect the contribution of leukocyte subsets to the pathophysiology of stroke.

Improving Current Models
Culture Conditions: Rethinking the Status Quo
Although there have been a few attempts to mimic the complex architecture of the brain, such complex systems are not always required; however, mimicking physiological environments in terms of the chemical microenvironment and relevant culture conditions is crucially important.

Glucose: Are Cell Cultures Diabetic?
In vivo, plasma glucose can range from 5.5 to 7.8 mmol/L, whereas brain glucose levels have been reported ranging from 0.82 to 2.4 mmol/L. However, glucose levels found in cell cultures (designed for rapid cell growth) far exceed physiological levels, often containing >20 mmol/L glucose. In severe hyperglycemia, plasma levels may reach 15.2 mmol/L, and in hypoglycemia, may drop to 2.8 mmol/L, whereas brain levels only reach 4.5 mmol/L, or fall as low as 0.16 mmol/L. As such, neurons in culture are consistently exposed to >10× the levels found in vivo. High levels of glucose in standard media have been shown to negatively affect long-term neuronal viability and influence AMP-activated protein kinase signaling. Levels of other energy sources including lactate, pyruvate, fatty acids, and ketone bodies have been demonstrated to influence neuronal metabolism both in vivo and in vitro and should be considered when investigating neuronal energy crisis. Regardless of the disease state or system being modeled, if the aim of an in vitro study is to predict or investigate the physiological in vivo environment, then it is logical that physiologically relevant conditions are maintained. The most widely used media formula were designed many decades ago, and it may be that it is time for a rethink of the conditions under which cells maintained.

Moving Into the Third Dimension
In Edwin Abbott 1884 novella flatland, the third dimension is described from a world in which only 2 dimensions exist. In this imaginary world, a sphere would appear as a series
of changing size circles. By ignoring the third dimension in vitro, we may equally only be getting a slice of the bigger picture of cells’ behavior.

Most 3D platforms can be broadly categorized into 3 groups: (1) hydrogel-forming (such as alginate and collagen), (2) synthetic microporous scaffolds (such as poly(styrene scaffolds), and (3) scaffold-free formats (such as hanging drop preparations). Most headway with regards to 3D BBB, neurovascular unit and stroke models have been made with the use of hydrogels because cross linking can be adjusted to provide physiological densities and hydrogels can also be functionalized to provide biochemical cues. Cells grown in 3D microenvironments exhibit distinct phenotypes, often with greater physiological relevance. Peretz et al. have shown that hippocampal neurons and astrocytes grown in 3D have significantly lower mortality rates and a higher neuron:astrocyte ratio than is achievable in 2D cultures. These 3D cell cultures were also more resistant to nutrient deprivation, and astrocytes may be more easily maintained in a resting state, allowing for rapid activation in response to stimulation. Although this additional layer of complexity may not be suitable for all stroke models, the 3D microenvironment undoubtedly influences cell behavior accordingly many cell culture systems are moving in this direction.

Human Cells for a Human Disease: Conditionally Immortalized Cells and All Human Models

Conditional immortalization, with for example, a temperature-sensitive, or drug-induced gene expression technologies such as c-mycER™, allow the use of cells which maintain their original phenotype, but with the convenience and reproducibility of a cell line. Amemori et al. have used this technology to produce the SPC-01 cell line from human neural stem cells, which are currently undergoing testing in a clinical trial for stroke. Such cells have not yet been used for stroke modeling but may provide a valuable tool in future research. Recent advances in iPSC technology have enabled study of not only monogenetic diseases but also sporadic and multifactorial diseases and may provide in vitro researchers with much of the experimental power that transgenic animals have granted in vivo research. Importantly, such technological advances are also allowing for more widespread use of all human systems. Indeed, iPSCs may pave the way for in vitro stroke models that are not only of human origin but also from a single donor. The benefit of all single donor systems is evident from the

**Figure 2.** Microfluidic blood–brain barrier (BBB) models. Examples of experimental microfluidic devices designed to model the BBB. A, Diagram showing a top view of the SyM-BBB microfluidic BBB model. Micropillars are used to separate a basolateral central reservoir to which astrocytes or astrocyte-conditioned media is added. Brain endothelial cells (BECs) are seeded in the peripheral channels, whereas the micropillars allow basolateral interaction. Reprinted from Prabhakarpandian et al. with permission of the publisher. Copyright ©2012, The Royal Society of Chemistry. B, The microfluidic BBB (μBBB) model uses a porous membrane to separate basolateral cells from BECs, inbuilt electrodes allow for transendothelial electrical resistance (TEER) measurement across the BEC monolayer. Reprinted from Booth and Kim with permission of the publisher. Copyright ©2012, The Royal Society of Chemistry. PDMS indicates polydimethylsiloxane.
work of Reed et al. who reveal that assays used to detect cytokine storms often fail because of mismatch between ECs used (often human umbilical vein cells) and the isolated leukocytes (from healthy adults). Their use of an autologous bioassay was able to respond to biologics, such as TGN1412, in a way that accurately reflects the clinical situation, thus providing great predictive power. In light of the above evidence, and considering many stroke models mix cells from different species, all human systems should be promoted for the modeling of human disease.

**Incorporating Physiological Flow Into Complex Models**

Exposure of ECs to physiological shear rates has been shown to increase longevity and inhibit cell cycle, influencing cell genotype and phenotype. Indeed, the importance of shear stress on cellular responses is particularly evident when there is a temporary loss of shear stress to ECs, as might occur during ischemic stroke, which causes cytokine release and BBB permeability. The presence of leukocytes was also found to compound these effects. It should be noted that these experiments simply used cessation of flow, with constant glucose and oxygen. Using models that not only deprive oxygen and glucose but also flow may provide the most relevant pathological stimulus to study mechanisms of stroke pathology.

**Microfluidic Systems**

Photolithographic etching techniques developed to fabricate computer microchips have since been adopted by biologists to develop micropatterned devices for cell culture. Such techniques provide new opportunities to mimic tissue architecture at the micro- and nanoscale.

The microfluidic BBB model is one of the first microfluidic systems to be applied to neurological research. Essentially a 2-layer (luminal and abluminal) flow chamber model contains built-in Ag/AgCl electrodes for TEER measurement. This

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**Figure 3.** Systems for modeling neuronal architecture in vitro. **A**, A microfluidic-based culture platform to direct axonal growth of central nervous system neurons; 2 chambers (yellow and black) are connected via subcellular diameter channels which allow axon growth between chambers and polarization of axonal and somal sides. Reprinted from Taylor et al. with permission of the publisher. Copyright ©2011, Macmillan Publishers Ltd. **B**, A microfluidic chip enabling for multiplexed electrophysiological experiments on cultured neurons: microfluidic junctions between the main cell culture chamber and the lateral recording capillaries allows for patch clamp recordings of cell (green) activities. Reprinted from Ionescu-Zanetti et al. with permission of the publisher. Copyright ©2005, National Academy of Sciences. **C**, The use of vacuum soft lithography to pattern biomolecules and direct neuronal adhesion and polarization: adhesion molecules are patterned on a glass coverslip using a microfluidic template to allow for directed cell growth. Reprinted from Nevill et al. with permission of the publisher. Copyright ©2010, The Royal Society of Chemistry. **D**, The artificial synapse, a microaperture allows for delivery of picoliter volumes of neurotransmitter to neuronal dendrites enabling detailed investigation of synaptic activity. Reprinted from Peterman et al. with permission of the publisher. Copyright ©2003, John Wiley and Sons Inc.
model allows the establishment of BBB properties in a short timeframe and uses fewer reagents than traditional models. Other models such as the SyM-BBB have also been developed using microfabricated pillars rather than porous membranes \(^5\) (Figure 2).

Microfluidic fabrication techniques have also been used to model neuronal architecture (Figure 3), from guiding axons via channels to establish axonal polarization\(^52\) and improved capillary-free electrophysiological devices,\(^53\) to the complex patterning of adhesion substrates to create artificial neuronal networks.\(^54\) Furthermore, Peterman et al\(^5\) have recently created an artificial synapse, in which cultured neurons may be stimulated by the picoliter release of neurotransmitters. Such patterning of adhesion substrates to create artificial neuronal networks is amenable to automation and as such may allow for complex HT screening assays, which is currently at odds with physiological relevance.\(^25\)

**Conclusions**

Although current in vitro systems have a limited capacity to replicate the complex situation that occurs in stroke, when appropriately used for a specific research question, in vitro systems are a highly useful research tool to help understand basic biochemical and cellular mechanisms. With the increased availability of human cells, the development of new technologies and more physiologically relevant models, in vitro systems may provide not only a HT low-cost base for the drug discovery pyramid but also composite all human assays at the top, supplementing in vivo research to improve clinical translation.

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**Disclosures**

None.

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