Modeling Ischemic Stroke *In Vitro*: The Status Quo and Future Perspectives

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Introduction

Stroke is a devastating disease accounting for 5.5 million deaths annually worldwide[1]. Despite significant preclinical and clinical investigations, with more than 1026 candidate neuroprotective stroke drugs investigated and nearly 200 clinical trials, no effective therapy other than tissue plasminogen activator (TPA) has been approved[2]. The reasons for these failures are numerous. Recent Stroke Treatment and Academic Roundtable (*STAIR*) 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 modelling 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 vs. 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, due to availability of genetically altered strains. However, around 70 million years of evolution separate humans from rodents and just a 10% difference in genome implies that ~ 3,000 genes differ[3]. Aside from macro structural discrepancies, a number of cellular and molecular differences exist. For example, the expression levels of transporters and pumps that contribute to the BBB, and the functional diversity and abundance of astrocytes distinguish the human BBB from the rodent[4]. Duration of excitotoxicity, has also been demonstrated to differ significantly between humans and mice[5].

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Considering the large inflammatory component of stroke pathology, it is also important to note differences in immune biology between species. A number of important immune signaling molecules (IL-8, CXCL7, CCL18, MCP-4 and CCL24/CCL26) are expressed in humans but not mice, while CCL6, CCL9, and MCP-5 are present in mice but not humans[3]. As such, the possibility of therapeutic targets being present in humans, but not 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 six 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[6]. 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[6, 7]. Thus, human *in vitro* systems may provide highly valuable information as to a drug's safety whilst also allowing for cost effective high-throughput screening assays (HTSAs)[8]. However, of 100,000 studies on stroke investigated in 2011, only 5 were performed in human *in vitro* systems[8]. 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 *et al.,* have previously compared a number of chemical and enzymatic methods against oxygen glucose deprivation (OGD) with reperfusion (OGDr) in a renal epithelial cell model[9], 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 GOX/CAT system consisting of glucose oxidase (GOX) and catalase (CAT) and 2-deoxyglucose (an isomer of L-glucose, which cannot be metabolized). These methods were compared to OGD for 1h followed by 3h reperfusion. Antimycin mediated cell injury was found to be the most reproducible, whilst OGD required a much longer time to produce cell injury[9].

While chemical and enzymatic methods may be suited to high throughput (HT), due to 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–glucose deprivation (OGD)

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. While oxygen deprivation is usually used in combination with glucose deprivation, a number of *in vitro* studies have demonstrated hypoxia alone causes dramatic alterations in endothelial cell (EC) actin cytoskeleton and tight junction protein localization in BBB

models[10]. 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 modelling 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 4–8h with 2mM glucose and in>24h with 20mM glucose (a concentration typical of most classical media)[11]. GD alone is rarely used to model stroke and over 4h does not significantly influence survival of rat cortical neurons in culture[12]. However, in organotypic mouse hippocampus cultures, GD alone for 2.5h resulted in significant CA1 cellular damage[13]. Such discrepancies may be explained by the relative sensitivity of CA-1 neurons and highlights the importance of using heterogeneous cell populations.

Combining oxygen and glucose deprivation, causes primary neurons to undergo acute cell body swelling within less than 60min followed by apoptotic[14] and excitotoxic necrotic cell death[15]. In OGDr experiments, neuronal degeneration occurs over several hours, despite return to standard culture conditions[11], as is consistent with observations of *in vivo* I/R injury. OGD in primary mouse cortical neurons is also associated with a large increase in extracellular glutamate concentration[11] 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 (NMDA) or glutamate[16]. 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 table 1.

Brain slices

The brain slice method utilizes a thin slice of brain tissue (usually ~400μm), allowing for in-depth probing of neuronal circuitry. The slice is perfused with artificial CSF (aCSF), allowing rodent slices to be maintained for up to 12h. Removing glucose and replacing oxygen with nitrogen in this solution provides global OGD, causing neuronal and astrocyte depolarization within 10min in rat cortical slices[17]. The benefit of preserving architecture is that different vulnerabilities of neuronal cell types may be assessed[17]. Additionally, neuronal effects of ischemia can be separated from cerebrovascular influences[17]. Richard *et al*., have built upon this model to provide focal OGD achieved by perfusing OGD aCSF over a brain slice in a focal stream, while the remainder of the slice is perfused with normal aCFS. 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[18].

A key benefit of *in vitro* systems is the opportunity to work with human cells, as such Werth *et al*., utilized 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[19].

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 typical stroke patient.

While 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 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 due to lack of extrinsic afferent and efferent signals *in vitro*[20]. 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 endothelial cell (BEC) primary cultures, where trans-endothelial electrical resistance (TEER) is comparatively high compared to cell lines[21]. 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[22]. 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[23], thus application to HT is limited. Furthermore neurological research is particularly limited, due to the fact that once terminally differentiated into mature neurons, these primary cells can no longer replicate. On the other hand, human blood samples are readily available as such isolated primary leukocytes and platelets may be utilized 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 immortalised cell lines is the ready supply of human cells suitable for use in HTSAs. Immortalized human microglia cells have been produced e.g. 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 (reviewed by Haile *et al.*,)[24]. 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 concidered, 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 four-year-old female 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[25].

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 (reviewed by Selvaraj *et al.*,)[26]. 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[27]. Furthermore protocols for iPSC cell differentiation to neurons are improving to give near 100% purity of neurons[28]. iPSCs have been utilized to model a number of diseases for drug screening, and since 2008 have also been used in modeling neuronal disease[29]. 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 (MS). While stroke is a multifactorial disease, mendelian stroke syndromes do exist, such as Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy (CADASIL)[30]. 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 *et al.*,[23]. Such models generally consist of a two-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 3D models allowing co-culture have also more recently been developed[31] (Figure 1B).

Building on BBB models: The neurovascular unit (NVU)

BBB models using BECs alone, suffer from low TEER and improper transporter and junctional protein localization compared to those found *in vivo*[32]. However it is now recognised that the BBB is supported by multiple heterotypic cell interactions as part of a NVU, involving astrocytes, pericytes, microglia, neurons and also extracellular matrix components.

Astrocytes

Astrocytes, the most abundant glial cells in the brain, whose endfeet cover 99% of the vascular surface and contribute to the unique properties of BECs and thus BBB integrity[25]. In agreement with this hypothesis, co-culturing astrocytes with BECs significantly increases TEER and enhances the expression of transporters, BBB enzymes and tight junctions[25]. Astrocytes also actively participate in stroke pathology, and exposing BECs to media from primary astrocytes which have experienced hypoxia, stimulates inflammatory genes in BECs[33]. Astrocytes have also been shown to influence immune cell recruitment to BECs[34]. Such co-culture 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*[25, 35]. *In vitro* pericytes have also been shown to promote BBB characteristics in BECs[25]. 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 TNF-α promotes BBB compromise[25]. Such apparently contradictory results are likely due to the complex microglial phenotype and activation state. Kaushal and Schlicher recently used a novel *in vitro* model of penumbral damage to help delineate the microglial involvement in stroke[36]. In this model, neuron and astrocyte co-cultures undergo OGD to simulate the stroke core. Soluble factors from the stressed co-cultures are then used to activate the microglia. These microglia are then incubated with naive neuron/astrocyte co-cultures to reveal how microglia may damage healthy tissue[36]. 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. While 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[25] suggesting that inclusion of neuronal cultures in BBB models may provide useful mechanistic insights into ischemia-induced BBB breakdown.

Extracellular matrix

The importance of extacellular matrix is exemplified by Tilling *et al*., using porcine BECs. These experiments with various combinations of matrix coatings demonstrate significant effects on BEC permeability[37].

Beyond the brain: Recruitment of circulating cells

While 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 a number of 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 utilized 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[38]. This is consistent with neutrophil migration *in vivo* causing vasogenic cerebral edema and BBB breakdown following stroke. OGD has also been shown to up-regulate BEC adhesion molecules, chemoattractive factors, and neutrophil binding under static conditions[33]. 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: Re-thinking the status quo

While there have been a few attempts to mimic the complex architecture of the brain, such complex systems are not always required, however mimicking physiological environment in terms of the chemical microenvironment and relevant culture conditions is crucially important.

Is 'Hypoxia' in vitro, normoxia in vivo?

Oxygen is critical to cell function, too little and cells will die whilst too much enhances free radical production. Standard incubator conditions often utilize 5% CO_2 to maintain pH, however oxygen is rarely controlled for. Circulating arterial blood typically contains 10.5–13% (80–100mm Hg) oxygen and most organs typically function at 2–8% O_2 (19–70mm Hg)[39]. However, atmospheric oxygen levels are often referred to as "normoxic" despite being substantially higher than those *in vivo*. While many ODG studies use relevant hypoxia levels (<2%), relatively few return to normoxia (2–5%), instead returning them to atmospheric levels. Thus the oxygen level changes in OGD are more extreme than found *in vivo*. A number of reports have demonstrated exposure to physiological oxygen conditions (2–5%) to increase survival, proliferation and dopaminergic differentiation of neurons in culture[40]. In addition to altering basal functioning, high oxygen levels also alter cellular response to challenge, with neurons cultured at atmospheric oxygen being resistant to HIV induced cell death[39]. It is thus plausible that these cells are resistant to the oxidative stress produced in stroke, considering they have grown in an oxidative environment prior to challenge. As such, the influence of oxygen levels should be taken into account when interpreting data from *in vitro* experiments.

Glucose: Are cell cultures diabetic?

In vivo, plasma glucose can range from 5.5 to 7.8mM, while brain glucose levels have been reported ranging from 0.82 to 2.4mM[41], [42]. However, glucose levels found in cell cultures (designed for rapid cell growth) far exceed physiological levels, often containing >20mM glucose. In severe hyperglycemia plasma levels may reach 15.2mM, and in hypoglycemia, may drop to 2.8mM, while brain levels only reach 4.5mM, or fall as low as 0.16mM[41]. As such, neurons in culture are consistently exposed to >10 times the levels found *in vivo*. High levels of glucose in standard media have been shown to negatively affect long term neuronal viability and influence AMPK signaling[41]. 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*[41] 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 a number of decades ago and it may be that it is time for a re-think of the conditions under which cells maintained.

A glimpse into the future

Taking the above considerations into account to improve the physiological relevance, new technologies and understandings may allow for the development of newer models with increased predictive power and providing more faithful representations of pathophysiological microenvironment.

Moving into the third dimension

In Edwin Abbott Abbott's 1884 "novella flatland", the 3rd 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 3rd dimension *in vitro* we may equally only be getting a slice of the bigger picture of cells' behavior.

Most 3D platforms can be broadly categorised into three groups: 1) Hydrogel-forming (such as alginate and collagen), 2) Synthetic microporous scaffolds (such as polystyrene scaffolds) and 3) Scaffold-free formats (such as hanging drop preparations)[43]. Most headway with regards to 3D BBB, NVU and stroke models has been made with the use of Hydrogels, because cross linking can be adjusted to provide physiological densities and hydrogels can also be functionalised to provide biochemical cues[43]. 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[44]. 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[45]. While this additional layer of complexity may not be suitable for all stroke models, the 3D microenvironment undoubtedly influences cell behavior and accordingly a number of 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^{TAM}, 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[46]. Such cells have not yet been utilised for stroke modelling 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[8]. 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 work of Reed *et al.*, who reveal that assays used to detect cytokine storms often fail due to mismatch between ECs used (often human umbilical vein cells) and the isolated leukocytes (from healthy adults)[47]. 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 predicative power[47]. In light of the above evidence, and considering a number of stroke models mix cells from different species, 'all human systems' should be promoted for the modelling 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[48]. 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[49]. It should be noted that these experiments simply used cessation of flow, with constant glucose and oxygen. Utilizing 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 micro-patterned devices for cell culture. Such techniques provide new opportunities to mimic tissue architecture at the micro and nano scale.

The microfluidic BBB (μBBB) model is one of the first microfluidic systems to be applied to neurological research. Essentially a two-layer (luminal and abluminal) flow chamber the model contains built-in Ag/AgCl electrodes for TEER measurement[50]. This 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[51] (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*. have recently created an artificial synapse, in which cultured neurons may be stimulated by the pico liter release of neurotransmitters[55]. Such devices have exciting implications for the study of excitotoxicity and neuronal interaction within networks. Microfluidic devices are also currently being used to investigate thrombosis and microvascular occlusion in hematologic disease. Tsai *et al.,* have utilized a vascular tree of "endothelialized" microfluidic channels to investigate hemodynamic effects on thrombosis in blood samples from patients with Sickle Cell Disease[56]. Investigations of thrombosis in microfluidic devices resembling the NVU might in future be utilized to simultaneously investigate thrombolytic and neurological effects of test. A number of microfluidic devices are commercially available and while currently low throughput, such devices are also amenable to automation and as such may allow for complex HTSAs which is currently at odds with physiological relevance[25].

Conclusions

While current *in vitro* systems have a limited capacity to replicate the complex situation that occurs in stroke, when appropriately utilized 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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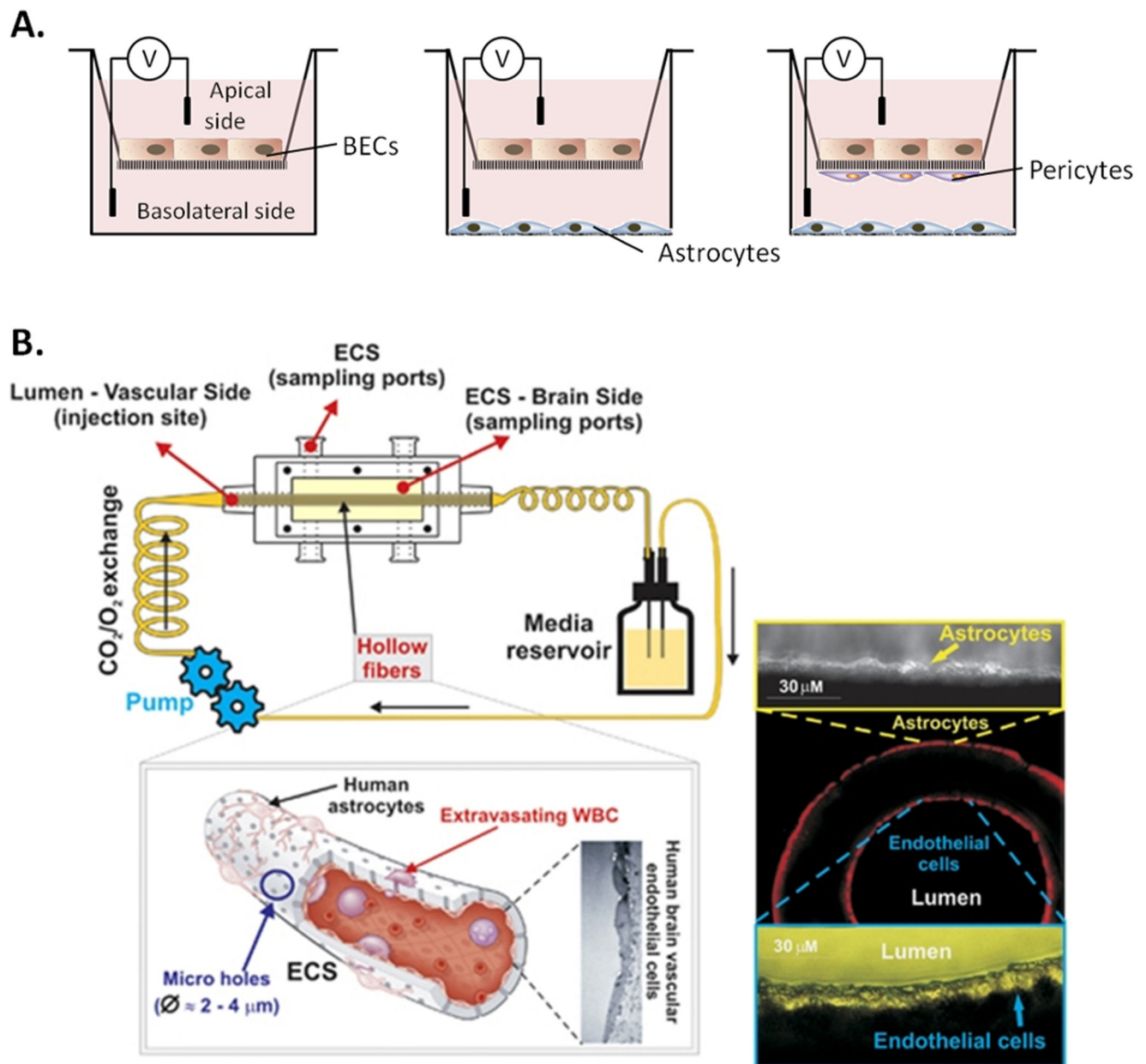
Figure 1. Currently used blood brain barrier (BBB) models

A) Diagrams demonstrating the varying complexities of transwell BBB models showing direct and indirect co-culture 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 co-cultured in the basolateral side of the well to enhance BBB properties, whist 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 3D co-culture of BECs and astrocytes whilst also allowing luminal flow to investigate leukocyte transmigration (Reprinted by permission from Macmillan Publishers Ltd; Journal of Cerebral Blood Flow & Metabolism[31] Copyright 2011). ECS, extraluminal space.
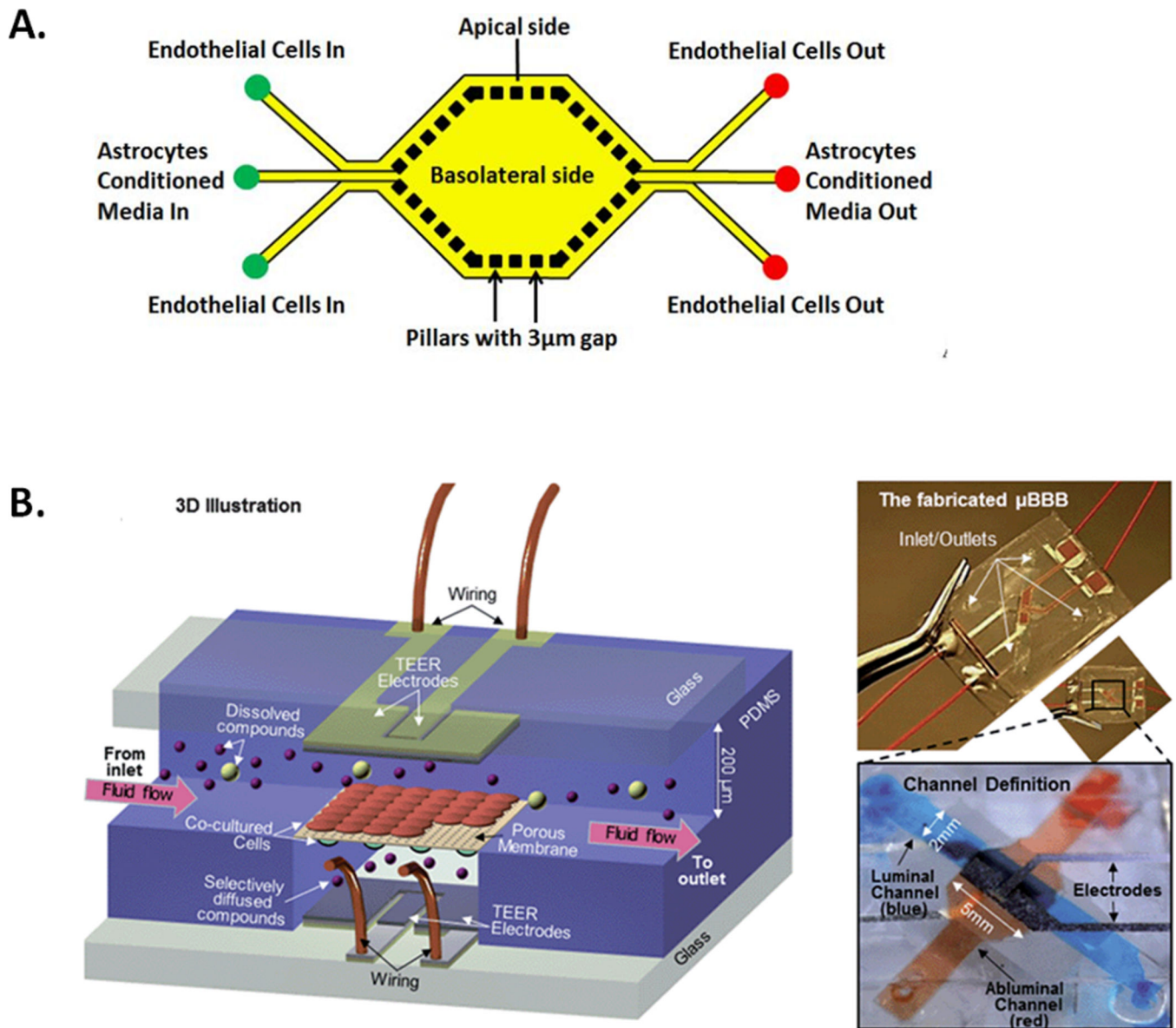
Figure 2. Microfluidic BBB models

Examples of experimental microfluidic devices designed to model the BBB A) Diagram showing a top view of the SyM-BBB microfluidic BBB model. Micro-pillars are used to separate a basolateral central reservoir to which astocytes or astrocyte-conditioned media is added. BECs are seeded in the peripheral channels whilst the micropillars allow basolateral interaction.[51] B) The μBBB model, uses a porous membrane to separate basolateral cells from BECs, inbuilt electrodes allow for TEER measurement across the BEC monolayer[50]. (Reproduced in part from Ref [51] and Ref [50] with permission of The Royal Society of Chemistry)
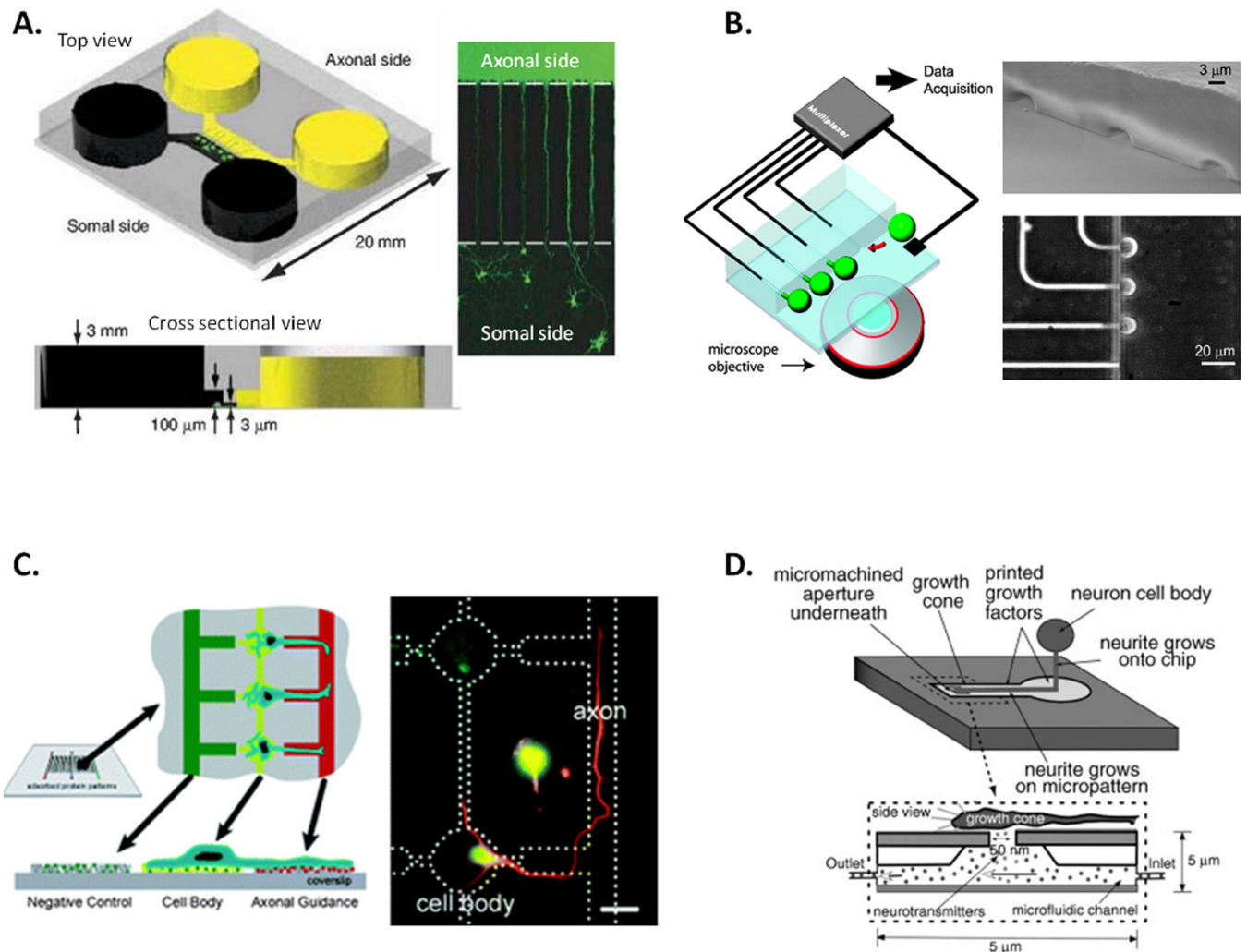
Figure 3. Systems for modeling neuronal architecture *in vitro*
A) A microfluidic-based culture platform to direct axonal growth of CNS neurons; two chambers (yellow and black) are connected via sub-cellular diameter channels which allow axon growth between chambers and polarization of axonal and somal sides (Reprinted by permission from Macmillan Publishers Ltd; Nature Methods [52] Copyright 2011). B) A microfluidic chip enabling for multiplexed electrophysiological experiments on cultured neurons: microfluidic junctions between the main cell culture chamber and lateral recording capillaries allows for patch clamp recordings of cell (green) activities (Reprinted from Ref [53] with permission from PNAS Copyright 2005 National Academy of Sciences, U.S.A). 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 (Reproduced in part from Ref [54] with permission of The Royal Society of Chemistry). D) the artificial synapse; a micro-aperture allows for delivery of pico liter volumes of neurotransmitter to neuronal dendrites enabling detailed investigation of synaptic activity (Reprinted from Ref [55] with permission from John Wiley and Sons Inc. Artificial Organs. Copyright 2003).

Table 1

Comparison of suitability of cellular platforms for *in vitro* neurological research

Cellular platform	HT ready	Availability of human cells	Physiological relevance	Structural complexity modelled	Possible application to stroke research	Comments
Brain Slice	No	Extremely limited	High	To a high degree	OGD, chemical ischemia, excitotoxicity	Tissue damage during preparation may induce abnormal functioning
Organotypic cell culture	No	Limited	High	To some degree	OGD, chemical ischemia, excitotoxicity	Tissue damage during preparation may induce abnormal functioning
Primary cells	No	Limited	High	Artificial arrangement required	OGD, chemical ischemia, excitotoxicity BBB models, thrombosis and leukocyte responses	Limited proliferative capacity
Cell lines	Yes	Unlimited	Low	Artificial arrangement required	OGD, chemical ischemia, excitotoxicity BBB models	Oncogenes present and high passage may limit physiological relevance
Embryonic stem cells	Partial	Extremely limited	Potentially High	Artificial arrangement required	OGD, chemical ischemia, excitotoxicity, BBB models	Limited use due to ethical issues
iPSCs	Yes	Potentially unlimited	Potentially High	Artificial arrangement required	OGD, chemical ischemia, excitotoxicity, BBB models	Single donor models of the relevant 'at risk' populations are possible