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Modern Methods and Materials
for Modeling Brain Tissue
and Blood-Brain Barrier *In Vitro*

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Abstract. Neurovascular unit (NVU) is an ensemble of brain cells (cerebral endothelial cells, astrocytes, pericytes, neurons, and microglia), which regulates processes of transport through the blood-brain barrier (BBB) and controls local microcirculation and intercellular metabolic coupling. Dysfunction of NVU contributes to numerous types of central nervous system pathology. NVU pathophysiology has been extensively studied in various animal models of brain disorders, and there is growing evidence that modern approaches utilizing in vitro models are very promising for the assessment of intercellular communications within the NVU. Development of NVU-on-chip or BBB-on-chip as well as 3D NVU and brain tissue models suggests novel clues to understanding cell-to-cell interactions critical for brain functional activity, being therefore very important for translational studies, drug discovery, and development of novel analytical platforms. One of the mechanisms controlled by NVU activity is neurogenesis in highly specialized areas of brain (neurogenic niches, NNs), which are well-equipped for the maintenance of stem/progenitor cell pool and proliferation, differentiation, and migration of newly formed neuronal and glial cells. Specific properties of brain microvascular endothelial cells, particularly, high content of mitochondria, are important for establishment of vascular support in NVU and NNs. Metabolic activity of cells within NNs and NVU contributes to maintaining intercellular communications critical for the multicellular module integrity. We will discuss modern approaches to

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development of optimal microenvironment for in vitro BBB, NVU and NN models with the special focus on neuroengineering and bioprinting potentials.

Keywords: brain, in vitro blood-brain barrier model, brain-on-chip models, scaffold.

Современные методы и материалы моделирования тканей мозга и гематоэнцефалического барьера *in vitro*

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Аннотация. Нейроваскулярная единица (НВЕ) – это совокупность клеток головного мозга (церебральные эндотелиальные клетки, астроциты, перициты, нейроны, микроглия), которые регулируют процессы транспорта через гематоэнцефалический барьер (ГЭБ), контролируют местную микроциркуляцию, межклеточную метаболическую связь. Дисфункция НВЕ способствует возникновению многих типов патологии центральной нервной системы. Патофизиология НВЕ широко изучена на различных моделях заболеваний мозга на животных. В настоящее время появляется все больше свидетельств того, что современные подходы с использованием моделей in vitro наиболее перспективны для оценки межклеточных коммуникаций внутри НВЕ. Разработка сосудисто-нервных единиц на чипе или ГЭБ на чипе, а также 3D НВЕ и модели ткани мозга обеспечивают новые подходы к пониманию межклеточных взаимодействий, критических для функциональной активности мозга, поэтому они очень важны для трансляционных исследований, открытия лекарств и создания новых аналитических платформ. Одним из механизмов, который контролируется активностью НВЕ, является нейрогенез в узкоспециализированных областях

мозга (нейрогенные ниши, НН), которые служат источником для поддержания пула стволовых/ прогениторных клеток, пролиферации, дифференциации и миграции новообразованных нейронов и глиальных клеток. Специфические свойства эндотелиальных клеток микрососудов головного мозга, в частности высокое содержание митохондрий, важны для создания сосудистой поддержки при НВЕ и НН. Метаболическая активность клеток внутри НН и НВЕ способствует поддержанию межклеточных коммуникаций, критически важных для целостности многоклеточного модуля. В работе обсуждаются современные подходы к разработке оптимальной микросреды для in vitro моделей ГЭБ, НВЕ и НН. Особое внимание уделено перспективам нейроинженерии и биопечати.

Ключевые слова: мозг, модель гематоэнцефалического барьера in vitro, модели «мозг на чипе», каркас.

Introduction

Current achievements in neurobiology and biotechnology brought us novel approaches to deciphering the mechanisms underlying brain activity. Growing evidence indicates that application of new protocols of brain tissue reconstruction in vitro might shed light on molecular and cellular events determining the phenomenon of brain plasticity. Development of in vitro brain-on-chip models, in vitro blood-brain barrier models, or cerebral organoids leads to significant progress in basic, translational, and clinical neurosciences (Kilic et al., 2016; Bang et al., 2019).

In vitro brain tissue models suitable for application in pharmacology, toxicology, stem cell research, etc., should be based on the minimally acceptable functionally competent brain unit called the neurovascular unit (NVU) (Muoio et al., 2014). The NVU is an ensemble of brain cells (cerebral endothelial cells, astrocytes, pericytes, neurons, and microglia), which regulates processes of transport through the blood-brain barrier (BBB) and controls local microcirculation and intercellular metabolic coupling. It is commonly accepted that dysfunction of NVU contributes to numerous types of central nervous system pathology including brain ischemia, neurodegeneration, neurodevelopmental disorders, depression, epilepsy, etc. (Iadecola, 2017; McConnell et al., 2017). Such changes are usually associated with the impairment of astroglia-driven metabolic regulation of neuronal and endothelial cells, cell-to-cell communications, development of neuroinflammation, and BBB breakdown (Tohidpour et al., 2017; Salmina et al., 2021). NVU pathophysiology has been extensively studied in various animal models of brain disorders, and there is growing evidence that modern approaches utilizing in vitro models are very promising in the assessment of intercellular communications within the NVU.

Multiple in vitro NVU and BBB models are currently available in 2D format (NVU on a dish or plate), 3D format (transwell system with inserts covered with permeable membranes; hydrogel-embedded models, etc.), or in microfluidic format (systems with more or less complex architecture of the chip allowing establishment of cell perfusion and reproduction of changes in BBB cells induced by fluid rheology, i. e. shear stress or reactive oxygen species production in endothelial cells) (Salmina et al., 2021). For example, microfluidic/microphysiological systems have been created to reproduce different brain tissue compartments: blood, brain, and cerebral spinal fluid (Alcendor et al., 2013). Moreover, recent years brought us new models based on cerebral organoids obtained from induced pluripotent cells (Jeong et al., 2020), which offer new approaches to constructing brain-on-chip or neurogenic niche-on-chip models.

Development of NVU-on-chip or BBB-on-chip and 3D NVU and brain tissue models suggests novel clues to understanding cell-to-cell interactions critical for brain functional activity, being therefore very important for translational studies, drug discovery, and development of novel analytical platforms (Alcendor et al., 2013; Maoz et al., 2018; Bhalerao et al., 2020). The chips used for NVU and BBB modeling are made from polydimethylsiloxane (PDMS), collagen, gelatin, fibrin, polycarbonate, polypropylene, polycaprolactone/graphene, polylactic acid, etc. (Osipova et al., 2018; Bhalerao et al., 2020; Mantecón-Oria et al., 2020). The main idea of choosing the material for a model is to mimic the extracellular matrix properties critical for NVU functions (basement membrane in the case of BBB), that is why numerous materials have been already tested for their compatibility with NVU/BBB cells. For instance, porous polycaprolactone/poly (D, L-lactide-co-glycolide) (PCL/PLGA) microfluidic perfusion

system was shown to serve as a vasculature network, and other types of NVU cells were co-cultured in a collagen matrix wrapping the vasculature network to produce the vascularized neural construct (Yue et al., 2020). Some models allow reproducing the gradient of molecules regulating cell development or proliferation, i. e. lactate embedded into photopolymerized gelatin scaffolds, as we showed before (Salmin et al., 2017). In other cases, microarchitecture of channels within the chip allows separating different parts of cells, i. e. neuronal soma and axon; therefore, more accurate modulation of cell activity can be performed (Bang et al., 2019).

The past decade opened new opportunities in modeling the brain tissue in vitro. Development of isogenic NVU/BBB models from induced pluripotent stem cells (iPSCs) might be useful for the generation of personalized brain tissue models suitable for diagnostic and therapeutic properties (Canfield et al., 2019). Application of 4D bioprinting promises novel approaches to reconstructing some elements of activity-dependent or stimuli-induced brain plasticity in vitro (Esworthy et al., 2019; Warren et al., 2021). However, modern in vitro NVU/BBB models have some technical difficulties that should be overcome. For example, obvious advantages of microfluidic in vitro NVU/BBB models like reproduction of «blood and cerebrospinal fluid flow» or achievement of higher integrity of the brain microvascular endothelial cells (BMECs) monolayer are diminished by another problem: culturing of NVU cell types on microfluidic chips changes their gene expression profiles caused by aberrant surface-to-volume ratios and substrate materials, as it was shown recently (Middelkamp et al., 2021). iPSCs-derived endothelial cells exhibited compromised expression pattern (Lu et al., 2021) and might be not applicable for the current BBB studies. Thus, to study the mechanisms that control functional competence

of NVU/BBB cells is of great importance for neurobioengineering and neurobiotechnology.

In general, placement of BBB cells in a transwell, hydrogel-based, or microfluidic platforms results in the formation of cell layers whose functional competence would depend on the combination of various factors: availability of nutrients and oxygen, preserved expression pattern of ion channels, transporters, enzymes, transcription factors and other signaling molecules, and the presence of regulatory growth factors and cytokines in the medium. Structural and functional integrity of the BBB could be further assessed using different approaches like measurement of the transendothelial electric resistance (to ensure establishment of integral barrier), analysis of metabolite levels in the extracellular space in different compartments of the model, and evaluation of the barrier permeability with easily identified compounds or complexes (i. e. Lucifer yellow, dextrans, liposomes, fluoresceins, and labeled xenobiotics) (Alcendor et al., 2013).

General characterization of functional and metabolic status of NVU

Identification of NVU as a self-supporting module in the brain means the association of all events recorded in it with changes in local microcirculation or permeability of the BBB (Fig. 1). In modern neurobiology, this is interpreted as the occurrence of several important (patho) physiological phenomena:

1) neuron-astroglial metabolic coupling: activated neurons stimulate astrocytes as glycolytically active cells that produce lactate, which is utilized by neurons, converted into pyruvate, and used in the Krebs' cycle to further ensure the operation of the mitochondrial electron transport chain (Mangia et al., 2009; Descalzi et al., 2019);

2) gliovascular control: an increase in the local concentration of extracellular lactate as a product of astrocytic metabolic activity leads to vasodilation needed for efficient oxygen supply in active brain regions (Mendrinos et al., 2008);

3) metabolic control of cerebral endothelial cells by pericytes and perivascular astroglia, enabling a change in the permeability (paracellular and transcellular) of the BBB, for the formation of a microenvironment in the tissue, which promotes the functional activity of neurons (Salmina et al., 2019);

4) mechanisms implying a local increase in the BBB permeability in the brain tissue, for instance, in neuroinflammation (associated
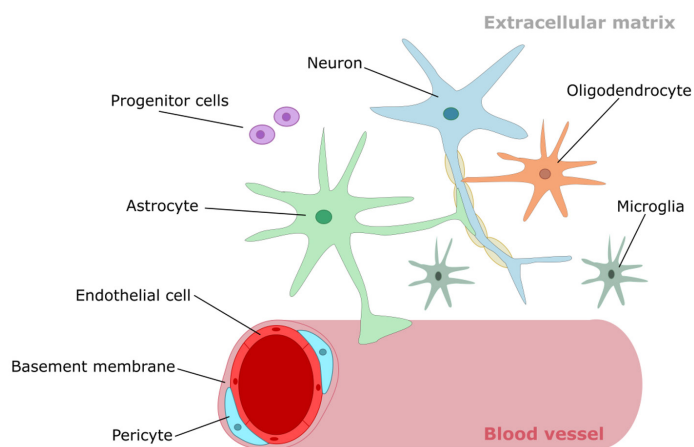

Fig. 1. Structure and composition of the neurovascular unit and the blood-brain barrier

with glial cells polarization), in neurogenesis (in neurogenic niches supporting the survival of neuronal stem and progenitor cells, or their activation followed by proliferation, differentiation, and migration), or in cerebral angiogenesis (based on the recruitment of endothelial progenitor cells and development of new vessels in active or affected brain regions) (Salmina et al., 2014; Tohidpour et al., 2017).

All of the above phenomena are based on quick and significant changes in the metabolic status of NVU/BBB cells, which are not studied in detail; however, the most critical characteristics of NVU cells should be taken into consideration when multicellular ensembles are going to be established in vitro.

BMECs are characterized by a higher content of mitochondria compared with endothelial cells in extra-brain localization; however, in a «resting» state, the main source of energy for them is glycolysis. Both glycolysis and oxidative phosphorylation (OXPHOS) are activated by pro-angiogenic stimuli in reparative cerebral angiogenesis or in activity-mediated cerebral angiogenesis (Malinovskaya et al., 2016). In addition, enhanced utilization of fatty acids further increases energy production in activated BMECs to support cell proliferation (Potente, Carmeliet, 2017). Disturbances in the glycolytic and mitochondrial activity of endothelial cells in cerebral microvessels lead to suppression of the angiogenic program and BBB breakdown and, presumably, disrupt the mechanisms of gliovascular control in the NVU (Chaitanya et al., 2014; Yetkin-Arik et al., 2019). Endothelial cells can respond to extracellular lactate of astroglial or peripheral origin due to expression of receptors for cell-derived lactate (GPR81 receptors) and monocarboxylate transporters (MCT), which ensure the uptake and release of lactate through the cell membrane. Alterations in GPR81 or MCT expression are usually associated with

BBB leakage and aberrant astrocyte-endothelial interactions, for instance, in neuroinflammation (Boitsova et al., 2018). Furthermore, disturbances in mitochondrial dynamics in endothelial cells (fusion and fission of mitochondria, mitochondrial biogenesis, and mitophagy) contribute to diminished angiogenesis and development of endothelial dysfunction (Shenouda et al., 2011; Xiang et al., 2021). In addition to excessive energy-producing metabolism, mitochondria in BMECs contribute substantially to generation of reactive oxygen species, which are considered now not only as inducers of oxidative stress, but also as cell signaling species (Itoh et al., 2006; Zhang, Gutterman, 2007). Moreover, endothelial cells are equipped with NADPH-oxidases (NOX), including dual oxidases (DUOX), which are the major sources of hydrogen peroxide and reactive oxygen species negatively affecting BBB permeability, but which might be also required in low concentrations to maintain the resistance of cerebral endothelium to the harmful effects of bacteria and viruses (Cahill-Smith, Li, 2014; Carvalho, Moreira, 2018; Anasooya Shaji et al., 2019). Another population of NOX-expressing cells in the NVU/BBB is represented by pericytes: their NOX4 plays an important role in supporting proliferative activity, which is necessary for angiogenesis and barriergenesis (Kuroda et al., 2014). In general, pericytes largely depend on their own glycolytic activity rather than on mitochondrial respiration. However, during hypoxia, pericytes can become glucose donors or even mitochondria donors for damaged perivascular astroglia (Trudeau et al., 2011; Salmina et al., 2019).

Perivascular astroglia, which is in close contact with BMECs and pericytes and with NVU neurons, is characterized by extremely high glycolytic activity and lactate production needed for efficient neuron-astrocyte metabolic coupling (Salmina et al., 2015). Release of lactate from

astrocytes ensures local hyperemia in active brain regions and supports neuronal ATP production via rapid conversion of lactate to pyruvate and acetyl-CoA in activated neurons. It is generally accepted that astrocytes are able to accumulate glycogen to quickly provide themselves and other NVU cells with glucose in the event of an insufficient supply of glucose from the blood (Matsui et al., 2017). Lactate produced by astrocytes is captured by neurons and utilized for mitochondrial activity; this is ensured by expression of MCTs for lactate and pyruvate in almost all of NVU cells, which support the influx and efflux of metabolites depending in the current needs of cells (Salmina et al., 2015). In addition to glycolytic activity, astrocytes can maintain their OXPHOS for a long time, even under unfavorable conditions, and even serve as donors of mitochondria for damaged neurons, for instance, in severe brain ischemia (Hayakawa et al., 2016). Suppression of mitochondrial activity in astrocytes leads to disruption of the mechanism of capture of excess glutamate supplied to the extracellular space by activated neurons, resulting in the development of excitotoxicity and neuronal death (Voloboueva et al., 2007).

Neurons have been traditionally considered as cells whose activity is mainly determined by mitochondrial energy production. The maximum number of mitochondria are concentrated in neurons in the perisynaptic zone, thereby reflecting the high demand of ATP and mitochondria-provided Ca2+ handling for synaptic neurotransmission and plasticity (Lee et al., 2018). Disruption of mitochondrial activity in neurons is an essential component of ischemia, brain damage, and chronic neurodegeneration, and modulation of mitochondrial dynamics is rather beneficial for the restoration of neurological deficits (Motori et al., 2020). Being stimulated, neurons are able to increase the production of ATP due to glycolysis and even to transfer excess lactate to other NVU cells (Díaz-García et al., 2017).

Microglia are characterized by complex metabolism affected by glia polarization: activated microglial cells depend on glycolytic ATP production rather than mitochondrial respiration. Activation of microglia promotes the assembly of intracellular inflammasomes and the expression of redox enzymes (NOX) involved in the generation of reactive oxygen species. Therefore, conversion of resting microglia to activated ones is accompanied by fragmentation of mitochondria followed by release of fragmented mitochondria to trigger astroglial activation and propagation of inflammation (Joshi et al., 2019). However, excessive production of reactive oxygen species facilitates elongation of mitochondria and might significantly affect mitochondrial dynamics (Katoh et al., 2017).

Oligodendrocytes are predominantly glycolytically active cells. They consume glucose and lactate during myelination, and glycolysis begins to dominate over the mitochondrial respiration soon after completion of the myelination program, probably to prevent excessive generation of reactive oxygen species in active mitochondria (Fünfschilling et al., 2012). Expression of the MCT in oligodendrocytes is necessary for axonal maintenance, therefore oligodendroglia-derived lactate is important for preventing axonal dystrophy (Fünfschilling et al., 2012).

Metabolic plasticity of NVU cells depends on extracellular matrix (ECM), particularly, in the case of BBB, and the basement membrane (BM) composition and porosity affect energy metabolism of cells. BM consists of several types of ECM proteins: collagen IV, laminin, nidogen, collagen XVIII, matrix metalloproteinases (MMPs), growth factors (i. e. VEGF) and cytokines, thrombospondins, fibronectins, lectins, etc. It has a thickness up to 200 nm and

numerous pores with the diameter from 5 nm to 8 μm (McConnell et al., 2017; Logsdon et al., 2021). In the parts of BMECs non-covered with pericytes, the BM contacts directly with perivascular astroglia and endothelial monolayer (McConnell et al., 2017). NVU cells are sensitive to BM and ECM composition, but the sensitivity mechanisms differ from each other. Very recent findings suggest that ECM may affect mitochondrial dynamics in adjacent cells: ECM stiffening promotes mitochondrial fusion and suppresses mitochondrial fission in a DRP1-dependent manner (Chen et al., 2021). This might be due to sensing of ECM composition by intracellular mitochondria connected via cytoskeletal proteins (De Cavanagh et al., 2009). Another type of functional coupling between ECM/BM and NVU cell metabolism is based on CD147 activity. CD147 (basigin) is expressed in neuronal, glial, and endothelial cells, and it promotes MMPs activation and amyloid precursor protein (APP) proteolysis due to functional association with gamma-secretase (Uspenskaya et al., 2018). Therefore, it is not surprising that aberrant expression of CD147 in NVU cells accompanies pathologically enhanced cerebral angiogenesis, development of neuroinflammation, and BBB breakdown in experimental Alzheimer's disease (Morgun et al., 2020). At the same time, CD147 regulates lactate transport associated with MCT transporters in the NVU (Uspenskaya et al., 2018). Therefore, activation of CD147 would result either in MMPs-mediated degradation of ECV/BM and loss of BBB integrity or in the activation of MCT1-, MCT4-mediated lactate and H+ efflux from glycolytically active cells (Kirk et al., 2000), presumably leading to prevention of intracellular accumulation of lactate and H+. The latter might be important either for lactate-driven changes in local microcirculation or for maintaining high level of glycolysis and NVU cell proliferation. It should be taken into consideration

that ECM and BM composition is greatly affected in brain diseases, i. e. in Alzheimer's type neurodegeneration (Thomsen et al., 2017). Therefore, application of BM analogues in the in vitro models of NVU/BBB/neurogenic niches (NN) could be inaccurate in reconstructing the impairments specific for the brain pathology.

Thus, in NVU/BBB in vitro models, cell metabolic activity should be supported with the optimal microenvironment, including soluble factors in media, and composition of ECM/BM or their analogues. Are these conditions fulfilled in the current in vitro models? It has now become clear that reproduction of the microenvironment supportive for efficient metabolic interactions between NVU/BBB cells could be achieved in rather complex systems like those based on 2 or more compartments, or in microfluidic chambers. For instance, in 3 coupled chips developed by (Maoz et al., 2018), mechanisms of influx across the blood–brain barrier (BBB) and to the brain parenchymal compartment and efflux across the BBB have been successfully reproduced. In that system, the BBB chip contained BMECs and pericytes, the brain chip contained mixed population of primary neural cells (dopaminergic, serotoninergic, GABAergic etc.), and in the coupled NVU chip, an endothelial medium (artificial blood) was placed to flow across the BBB chip, while neuronal medium (artificial cerebral spinal fluid) flowed through the perivascular compartment. The authors were able to develop functionally competent BBB and to preserve some parts (glycolysis, TCA, synthesis of GABA) of the linked metabolism of NVU cells, as it was confirmed by metabolomics (Maoz et al., 2018). Another approach was reported in (Huang et al., 2020), where a simpler system, made of primary astrocytes and BMECs, was used to get metabolomic profiling of endothelial cells in hypoxia. The authors demonstrated that astrocytes have greater capacity to rapidly

respond to the environmental stress whereas BMECs keep their metabolic activity at the baseline level and do not activate anaerobic ATP production, presumably to maintain the barrier intact. Therefore, BMECs appear to be more «metabolically rigid» compared to astrocytes in the NVU (Huang et al., 2020).

Cerebral organoids, which in recent years have been widely used to study brain activity and development, can be also considered as an in vitro NVU model. Development of brain region-specific cerebral organoids and assembloids (complex of various organoids) is a new step in the advancement of this technology (Jeong et al., 2020). However, there are some significant problems in the application of organoid models into translational studies. Actually, metabolism of cells within organoids is greatly compromised due to the absence of vessels (endothelial progenitor cells are not produced simultaneously with neuronal and glial cells because of their different origin). Therefore, the core cells within the organoids undergo hypoxic alterations leading to mitochondrial dysfunction,

apoptosis, and necrosis. Recent attempts at the development of vascularized organoids, i. e. based on iPSCs-derived BMECs, offer new opportunities in the construction of personalized BBB-on-chip models since these cells show some characteristics of BBB cells (Cakir et al., 2019; Kumarasamy, Sosnik, 2021).

Angiogenesis and neurogenesis at the NVU

As we have shown above, NVU cells effectively cooperate and support each other in their metabolic needs, particularly when they are activated. Two general plastic processes in the developing and adult brain – neurogenesis and angiogenesis – closely associate at the level of NVU (Sawada et al., 2014), and their efficacy depends on the integrity of NVU metabolism (Fig. 2).

It is well-known that in rodents, development of BBB begins at E10–17, the controlled permeability is formed by E21, but the development of tight junction machinery in BMECs continues even in the postnatal period.
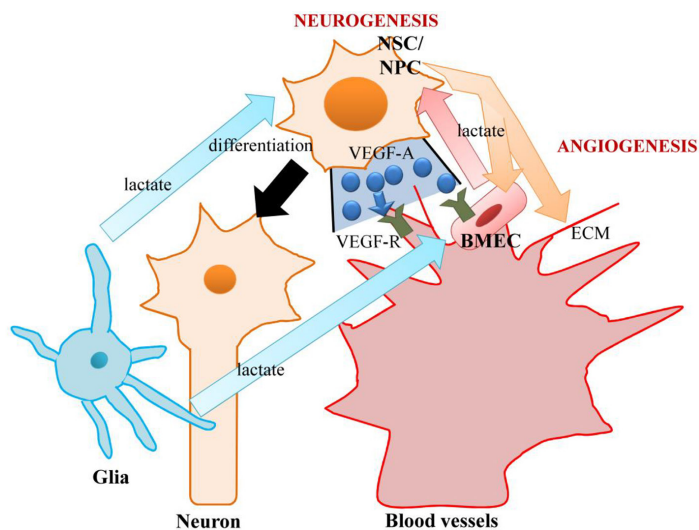

Fig. 2. Association of neurogenesis and angiogenesis at the level of neurovascular unit. Abbreviations: BMEC – brain microvessel endothelial cell, NSC – neural stem cell, NPC – neural progenitor cell, VEGF-A – vascular endothelial growth factor, VEGFR – VEGF receptor, ECM – extracellular matrix

In humans, BBB markers appear at the 8th week of embryogenesis, however, barriergenesis lasts up to 2–3 weeks of postnatal development (Malaeb et al., 2012). The most important point is that prenatal and early postnatal barriergenesis begins only after the establishment of the pool of neural stem cells (NSCs)/neural progenitor cells (NPCs) in the developing brain, and vascular and neural structures develop and mature simultaneously (Paredes et al., 2018). Molecules produced by developing immature neural cells stimulate specific patterning of sprouting capillaries and induce BBB characteristics in early BMECs (James, Mukouyama, 2011). Within the NVU, neuronal and astroglial cells affect angiogenesis in different modes. Specifically, loss of astroglial coverage results in excessive angiogenesis in the early postnatal brain (Ma et al., 2012). Thus, cerebral angiogenesis at the earliest stages of brain development tightly couples to synaptic activity and remodeling (Su et al., 2020). And vice versa, the germinal zone vasculature in the embryonic brain controls the balance between NPC self-renewal and production of new neurons irrespective of the oxygen-supplying ability of vessels (Tata et al., 2016). So, it is not surprising that the similar phenomenon is – at least partially – reproduced in the adult brain: functional activity of cells within the neurogenic niches (subventricular zone, SVZ, and subgranular zone, SGZ) depends on the preservation of vascular scaffold and local BBB permeability (high in the SVZ and low in the SGZ) (Pozhilenkova et al., 2017). In brain ischemia, loci with enhanced BBB permeability serve as multiple newly-established neurogenic niches along the ventricular system of the brain (Lin et al., 2015). The same outcome might be achieved by treatments affecting BBB integrity, i. e. transcranial focused ultrasound-induced reversible BBB breakdown stimulates hippocampal neurogenesis (Mooney et al., 2016).

How can the concept of tightly coupled neurogenesis and angiogenesis/barriergenesis be implemented in NVU modeling? Actually, the basic idea is to construct the reliable in vitro model of the neurogenic niche with the vascular support (Winkelman et al., 2021). In this context, appropriate ECM would greatly affect the reliability of the model since it was demonstrated that ECM composition controls self-renewal and prologeration of stem cells (Guilak et al., 2009). Application of various materials in the microfluidic models allowed identifying the most suitable conditions for integrating neurogenesis and angiogenesis: matrigel mixed gel promoted NSC differentiation into neurons, whereas capillary-like structures were formed in the fibrin-matrigel mixed gel by co-culturing BMECs and human mesenchymal stem cells (MSCs); then, a 3D NVU model, as a triculture system made of NSCs, BMECs, and MSCs, was constructed from neural and vascular compartments in the microfluidic device (Uwamori et al., 2017). Another approach was demonstrated in the spheroid BBB model: chitosan- or gelatin-based substrates were used to generate the 3D NSC/BMEC co-spheroids with pro-angiogenic potential (Han et al., 2019). We demonstrated that neurogenic activity of NSCs co-cultured with astrocytes and BMECs within the NN in vitro model implanted into hippocampus could be efficiently regulated by optogenetic stimulation of ChR2-expressing astroglia (Morgun et al., 2021).

The next step in the development of in vitro NN models is their application in regenerative neurology. Thus, considerable research has focused on bioprinting potential of NVU/NN models and cerebral organoids. One of the biggest challenges in this field is to create the «building blocks» that would resemble the structure of the real NVU or NN, and this depends on the availability of new materials as substituents of the ECM/BM (Potjewyd et al., 2018). The

following biomaterials are now tested for NVU/NN scaffolds in the context of 3D bioprinting technology: collagen, gelatin, fibrin, hyaluronan, and PEG (Potjewyd et al., 2018). Their physical and chemical properties can be modified via cross-linking, introduction of some modifying agents, or changing the porosity and stiffness. For instance, the presence of specific amino acid sequences (Arg–Gly–Asp (RGD) and Ile–Lys–Val–Ala–Val (IKVAV)) is important for integrin and laminin functions within the NVU ECM or BBB BM (Potjewyd et al., 2018). Decellularized natural ECM can be used for in vitro NVU and BBB modeling, particularly, for the development of vascular support of stem cell differentiation (Hoshiba et al., 2016), whereas poly-lactic acid-based scaffolds provide metabolic support of cells, particularly, for astrocytes (Pavia et al., 2019). Anyway, the technology of 3D and 4D bioprinting is a promising tool for NVU/BBB/ NN and whole brain tissue modeling (Fantini et al., 2019).

Conclusion

Despite the huge number of in vitro NVU/BBB models available at present, no physiologically realistic one has been constructed yet. All the models that are currently in use have significant limitations, mainly associated with the apparent difficulties in achieving the controlled and tunable microenvironmental conditions that could support the activity of NVU cells and mimic brain plasticity. Once this issue has been resolved, there will be considerable progress in the development of in vitro models suitable for personalized testing of drug candidates, studying the molecular mechanisms of brain development and function, developing neuromorphic systems, and using 3D bioprinting protocols in basic, translational, and clinical studies.

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