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# Recent Preclinical and Clinical Technological Advances Suitable to Unravel the Physiological and Pathological Status of the Blood Brain Barrier in Neurology

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## Abstract

The physiological or altered status of the blood brain barrier (BBB) is essential in multiple neurological conditions affecting the central nervous system (e.g. Alzheimer's disease, Parkinson's disease, epilepsy, multiple sclerosis, brain ischemia, stroke, chronic pain etc.). The aim of this mini-review is to describe briefly the latest technological advances that enable a more accurate analysis of the BBB integrity, without any particular focus on a certain pathology. Sophisticated methods to artificially recreate BBB's properties have been rapidly developed in the last decade, including cell-based, brain slice or structure-on-chip models. Such *in vitro* systems are certainly useful but the differences from the actual physiological and pathological BBB characteristics limit their scope. Recent advances of *in vivo* cerebrovasculature imaging techniques in living animals or in patients were also reviewed. Magnetic resonance imaging remains an important clinical and preclinical tool for investigating blood brain barrier disruption. In the animal models, the two-photon microscopy brain imaging technique is the most suitable *in vivo* method for the BBB analysis, despite its invasiveness compared to magnetic resonance imaging. Light sheet microscopy, and the emerging technique of selective plane illumination microscopy, are cutting-edge technologies with possible future applications in BBB investigation. Optical coherence tomography is also a very promising preclinical and clinical tool for the investigation of brain vasculature, and probably in the next future will be applied for BBB analysis. In conclusion, recent technological progresses are able to distinguish permeability changes of the blood brain barrier, but several pieces of puzzle are still missing in terms of translating information from benchside to bedside.

***Keywords:*** *Blood brain barrier; Brain endothelial cells; Brain cerebrovasculature; In vitro blood brain barrier models; In vivo brain vasculature imaging*

**Abbreviations:** BBB: Blood Brain Barrier; BVEC: Brain Vascular Endothelial Cells; CNS: Central Nervous System; DCE-MRI: Dynamic Contrast-Enhanced MRI; TEER: Trans Endothelial Electrical Resistance; LSM: Light Sheet Microscopy; MRI: Magnetic Resonance Imaging; μBBB: Microfluidic Blood Brain Barrier; OCT: Optical Coherence Tomography; P-gp: P-glycoprotein; SPIM: Selective Plane Illumination Microscopy; SyM-BBB: Synthetic Microvasculature Blood Brain Barrier; SPIO: Super Paramagnetic Iron Oxide; USPIO: Ultra Super Paramagnetic Iron Oxide; ZO-1: *Zonula Occludens*.

## Introduction

Blood brain barrier (BBB) is represented by the endothelium of the cerebral microvasculature and plays an essential role in maintaining the central nervous system (CNS) homeostasis [1]. BBB disruption has been described in several neuro-pathological conditions affecting the CNS, e.g. Alzheimer's disease [2], Parkinson's disease [3], epilepsy [4,5], multiple sclerosis [6], brain ischemia [7], chronic

pain [8] etc. Several preclinical and clinical efforts to develop novel *in vivo* and *in vitro* approaches that highlight physiological and pathological BBB properties in neurology are ongoing. The purpose of this mini-review is to briefly describe the latest preclinical and clinical technological advances in the BBB study.

***In vitro* BBB models**

An interesting alternative for preclinical studies of BBB is the use of *in vitro* models. Some advantages of these tools, such as versatility, possibility to control different compounds etc., make them very attractive. On the other hand, there are some drawbacks of this approach. A good model has to mimic as much as possible the *in vivo* organization and function of the BBB. This is not a trivial task. A few parameters can have a major influence on the success of this approach: type and source of cells, support of growing, timing of seeding, medium composition, presence of shear stress conditions, etc. In the last decade several models have been proposed in the literature with promising solutions for mimicking *in vivo* BBB characteristics. In a brief overview, there are several categories of models:

a) 'classical' co-cultures based on different cellular components, either primary cultures or immortalized cell lines,
b) brain slices cultures and
c) structure-on-chip [9, 10].

Additionally, there is an increased tendency of developing computational (*in silico*) models, but these will not be further discussed in this mini-review. The co-culture and structure-on-chip model are versatile, components being easily controlled. Meanwhile, the brain slice culture model accurately retrieves the BBB into a piece of tissue, but is more complicate to control and measure different parameters. These models have been developed in either as static (without shear stress) or dynamic (shear stress) systems [9].

Models using co-cultures initiated from a simple monolayer of brain vascular endothelial cells (BVEC) or a derived immortalized cell line as bEnd3 and evolved adding other cellular components as astrocytes, pericytes and neurons. In a complex static model, BVECs are growing on one side of the membrane insert and astrocytes on the other side and neurons (as a third component) are added on the well bottom [11]. The 'qualities' of this model are: possibility to perform permeability and transendothelial electrical resistance (TEER) measurements, and a physiologic-like expression of different functional markers, such as P-glycoprotein (P-gp) and *Zonula occludens* (ZO-1). Dynamic models use a thin porous capillary as a mechanical support for seeding the BVECs and astrocytes, where BVECs were seeded on the internal side and astrocytes on the external side of the capillary. In this way, a fluidic circuit can be designed to control the shear stress on the surface of the luminal side on the BVECs monolayer mimicking the blood flow conditions [12]. These have also the great advantage to get new insights concerning the role of each type of cells participating in the model and their influence on brain endothelium monolayer function. For example, it was evidenced the opposite role of pericytes and astrocytes in modulating BBB characteristics [13]. An extensive review dedicated to suitability of using different murine cell lines to recreate a valid BBB model has been recently published [14]. However, the closest cell-based models to the human BBB properties are containing in their structure human cell co-cultures [15].

The use of organotypic brain slices as a BBB model has been also proposed [10]. The epileptic-like seizures have been mimicked by kainate acid treatment in rat organotypic hippocampus slice cultures [16].

Beside the 'classical' cell-based co-culture models of BBB, there is a rapid development of structure-on-chip models (Table 1), such as microfluidic BBB (μBBB), BBB-on-chip, SyM-BBB (synthetic micro vasculature BBB) [9, 17-22] and there are also advanced models such as the of 'neurovascular unit–on–a–chip' and the 'human-on-a-chip' systems.

The possibility of testing drugs targeted against BBB is a key research aspect and novel insights could contribute to possible therapies of barrier reseal that would be highly relevant in translational neurology. Consequently, the future improvement of in vitro barrier models, that are reproducing essential physiological features, can represent a great benefit and a premise for creating preclinical *in vitro* test platforms.

| Model | Type of Model | Description | Advantages |
|---|---|---|---|
| Triple coculture [11,23,24] | Static | Coculture of brain endothelial cells in the presence of pericytes and astrocytes, or neurons and astrocytes | Possibility to perform permeability and TEER measurements P-gp and ZO-1 expression |
| Organotypic slice cultures [16,25] | Static | Organotypic slice cultures | An elaborated network of vessels is retained in culture in spite of the absence of blood flow<br>Application of calcein-AM either from the interstitial or from the luminal side resulted in different staining patterns indicating the maintenance of a barrier |
| Organotypic slice cultures [10] | Dynamic | Organotypic slice cultures with cannulation and perfusion of parenchymal arterioles | Incorporates haemodynamic variables (flow and pressure) into parenchymal arterioles resulting in the development of myogenic tone |
| Coculture of organotypic slices with endothelial cells [26] | Static | Coculture of cortical organotypic slices with brain endothelial cells | Allows the experimental analysis of TEER, tight junction protein localization, and live cell imaging of reactive oxygen species |
| µBBB [19,21,27,28] | Dynamic | Microfluidic blood-brain barrier which with a dynamic environment and a thin culture membrane (~10 µm) | Good TEER values<br>Reliable permeability assays<br>ZO-1 expression |
| SyM-BBB [20] | Dynamic | Plastic, disposable and optically clear microfluidic chip with a microcirculation sized two-compartment chamber (side-by-side apical and basolateral compartments) | Functional transporter assays Upregulation of tight junction molecules |

***Tabel 1:*** *Latest developments of in vitro BBB models.*

### *In vivo* cerebrovasculature imaging approaches

So far, we have considered the *in vitro* models that recreate as reliable as possible the physiological features of BBB. In this section, we will try to highlight the progresses done in the field of 'real' BBB, where it becomes extremely important to have high-resolution imaging techniques in order to identify the finest changes occurred at the cellular level in murine or human subjects. There is a significant difference between *in vivo* preclinical studies where it is also possible to use invasive methodologies (e.g. two-photon microscopy or intravital imaging), compared to the clinical approach where the high-resolution non-invasive investigation techniques are essential for a good neurological diagnosis.

Two-photon *in vivo* imaging has dramatically changed our approach for studying in situ the brain processes at cellular and subcellular level. The spatio-temporal resolution of two photon microscopy is the main advantage of this technique allowing imaging of dyes diffusion or cellular trafficking by BBB in real time. The leakage through BBB disruption induced by ultrasounds was nicely imaged in rats [29]. Real time imaging of the BBB endothelium was done by intravital fluorescence video microscopy using a spinal cord surgical 'window' and the multistep extravasation of T cells across BBB was investigated [30]. With all its advantages, the two photon microscopy needs particular caution when performing extended imaging experiments. Long term imaging (e.g. up to several months) might induce brain trauma, which is reflected in the activation of astrocytes and microglia [31].

In the next future, the goals of *in vivo* approaches targeting BBB will be to image several events simultaneously at high-resolution, to obtain good 3D reconstructions and to reduce the photo-damage of brain tissue.

The Light Sheet Microscopy (LSM) allows a fast image acquisition combined to a low photo bleaching level. In a confocal set-up, LSM was used for *in vitro* 3D imaging in fixed specimens of the entire mouse brain at the cellular resolution [32]. In a two-photon excitation set-up, LSM allows capturing 3D images at 200 μm depth in living specimens with a good contrast and resolution and has been used for *in vivo* 3D imaging of deep organs in living zebra fish [33]. We might expect in the next future that LSM will be applied on BBB investigation and will bright relevant insights on BBB permeability changes in living animals. A cutting edge technology emerging from LSM is the Selective Plane Illumination Microscopy (SPIM), which was already applied for fast functional imaging of multiple brain regions in intact zebrafish larvae [34] and would be a possible solution for high resolution fast imaging of BBB dynamics.

Optical Coherence Tomography (OCT) is a very promising approach for the *in vivo* brain imaging with a high impact on the brain vasculature study, but still not used for BBB permeability investigations [35, 36]. With a the great advantage of being completely non-invasive, OCT ensures a resolution close to intravital microscopy and a field of view similar to magnetic resonance techniques (see the last paragraphs of this section). In the last period, OCT was developed to the clinical investigation of the blood cerebro vascular flow by means of the Doppler effect [37].

Another *in vivo* approach to acquire information about BBB physiology and pathology is the use of genetic animal models. Several genetic models are available for the study of BBB. Just to date some of them, there were developed genetic models targeting endothelial function (tight junctions, transcytosis, efflux and influx transport, leukocyte adhesion), genetic models manipulating pathways that affect angiogenesis, CNS-specific angiogenesis, BBB maintenance and BBB aging, genetic models targeting pericyte and astrocyte function [38]. Among these genetic models, the cutting-edge technological advances enable the the genetic fluorescent labeling of particular molecules that are relevant for the BBB imaging, including the so-called optogenetic tools [14].

The gold method in CNS diseases clinical diagnosis and preclinical studies (living animals) is the Magnetic Resonance Imaging (MRI). Several articles have reviewed this topic [39, 40]. Here we will just point out the major advances in the last years that improved this technique for the special requests necessary to detect the BBB permeability changes in clinical neurology. The standard MRI technique that uses Gadolinium-DTPA as contrast agent is extensively used as diagnosis method in many CNS diseases. However, the sensitivity of method, related to the affinity of the contrast agent to the serum albumins and to the tissue clearance kinetics, proved to not be good enough, since the standard test of Evans blue shows BBB leakage areas that MRI- GaDTPA is not able to reveal (reviewed in [41]). In the last years a new generation of contrast agents raised, the fluorinate Gadolinium compounds (Gaf) that improved the MRI sensitivity. Having a high affinity to serum albumins and a longer resting time in labelled tissue these new contrast agents allow the MRI-Gaf to provide in situ data matching with the Evans blue histological results. No Gaf contrast agent was yet approved for clinical use, so the MRI-Gaf can be used only in preclinical research studies. An alternative to Gadolinium-based contrast agents is the use of iron nanoparticles [42]. Compared to GaDTPA and Gaf that diffuse in the blood stream and indicate the BBB leakage by transvasing in the CNS tissues, the paramagnetic functionalized nanoparticles can specifically bind to immune cells and allow visualizing the immunological reaction at the level of BBB. Possessing a high negative magnetic susceptibility, superparamagnetic iron oxide (SPIO) or ultra superparamagnetic iron oxide (USPIO) nanoparticles have a higher sensitivity that Ga-based contrast agents being more suitable for quantitative measurements. SPIO/USPIO were designed for the specific labeling of macrophages, allowing to follow the infiltration of these cells in CNS. One of the most impressive results using a combination of MRI-Gaf or DTPA and MRI-SPIO is to provide clear evidences for the fact that BBB leakage and inflammatory response are produced in different areas of BBB. It was proved in this way that the relationship between these common aspects in CNS pathologies is complicated, the simple paradigm of a BBB leakage that allows the immune cells transvasation in CNS area being limited, and that leukocytes can enter the nervous system independent from a disturbance of the BBB [43].

One of the most powerful MRI methods in assessing the BBB disruption is the Dynamic contrast-enhanced MRI (DCE-MRI) [40]. Lately, an ingenious coupling between DCE-MRI and focused-ultrasound induced BBB opening enables to exploit the resulted changes in blood flow and perfusion (due to ultrasound) in order to image local BBB permeability changes [44].

## Conclusion

The information of BBB functionality acquired by means of in vitro models is valuable, but its limitations should be careful considered for translational medicine applications. The same is true for in vivo imaging on animal models and to what limit the preclinical data could be extended to clinical imaging. Additionally, there is a great low inter-comparability between MRI imaging studies of BBB disruption in patients due to the variability in acquisition and/or analysis methods. Considering all these limitations, but also by counterbalance the huge technological progresses done in last decade, the BBB detailed functionality remains a challenge in neurology and drug-design.

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## Conflict of Interest

The authors have no financial interest or any conflict of interest to declare.

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