comprising brain microvascular endothelial cells (BMEC), astrocyte and pericyte by taking advantage of immortalized cell line utility that allows various experimental approaches.
Methods: A human BBB model was constructed using immortalized human BMEC (HBMEC/ci18), astrocyte (HASTR/ci35), pericyte (HPBC/ci37). The gene expressions were determined by qPCR and immunocytochemistry. The BBB functions were examined by determining transendothelial electric resistance (TEER), lucifer yellow (LY) permeability and P-glycoprotein (P-gp) bi-directional transport. In the permeability assay, compounds were quantified by LC–MS/MS system.
Results: HBMEC/ci18 co-cultured with HASTR/ci35 and HBPC/ci37 showed elevated TEER (2.0-fold), reduced LY permeability (0.7-fold) and increased P-gp function (1.4-fold) compared with mono-culture. In support of these findings, expression levels of multiple BBB-specific genes, including barrier formation, transporters and receptors, were increased as well as their typical cellular localization in co-culture model. Notably, we performed BBB permeability assays using a set of 9 compounds with known CNS permeability characteristics. Expectedly, CNS-positive compounds (memantine, diphenhydramine, propranolol and pyrilamine) displayed high permeability coefficient values (Pe) (522 ± 100 to $1398 \pm 324 \times 10^{-6}$ cm/s). In contrast, CNS-negative compounds (quinidine, desloratadine, rhodamine 123, LY, sodium fluorescein) showed low Pe (21 ± 11 to $161 \pm 31 \times 10^{-6}$ cm/s).
Conclusion: We successfully developed a functional and scalable human BBB model. We hope that such research efforts are likely to open up new possibility of quantitative prediction of human CNS action based on in vitro experiment.

A2

A novel reusable, versatile, microelectric organ-on-a-chip device to study blood–brain barrier functions

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Objective: Biological barriers-on-a-chip models are cutting edge microengineered devices, but only a few combine the crucial parameters to study transport mechanisms, drug delivery and pathologies. Our laboratory developed a microelectric device (Walter et al. 2016), which enables visual observation, transendothelial electrical resistance (TEER) and permeability measurements on several biological barriers. The objective of our study was to further improve the device to make it more user-friendly and add novel functions.
Methods: The device was built up from a porous cell culture membrane sandwiched between two layers of PDMS and a top and bottom plastic slide coated with gold electrodes. After an automatic feeding period when the cells became confluent, a peristaltic pump was used to circulate the cell culture medium to mimic the blood flow. To verify the integrity, TEER was assessed with custom electrodes connected to an EVOM2 device. The endothelial surface charge was measured using silver electrodes connected to the outlets of the device. To validate our biochip we cultured the hCMEC/D3 human brain endothelial cell line and the stem cell derived CD34+ human endothelial cells in co-culture with bovine pericytes.
Results: We improved and optimized the biochip by (i) redesigning the shape of the electrodes, (ii) using universal luer-outlets, (iii) introducing small screws around the edges of the biochip we eliminated the use of the adhesive glue and (iv) could disassemble and reuse the device. The resistance was measured in real time using a custom made application compatible with cell phones. In the biochip under flow conditions the TEER elevated significantly in both BBB models which was also confirmed by ZO-1 and β-catenin immunostainings. A gene expression study was performed to investigate the differences between static and dynamic conditions on brain endothelial cells. Moreover a novel measurement of surface potential was also introduced.
Conclusion: This novel in vitro device for BBB culture models provides users with a standardized, reliable and reusable platform to perform pathology and pharmacology experiments. With advancement of the electrode layout, TEER automation and surface potential measurement our device is a cutting edge invention in the barrier-chip field.
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A3

Absolute computed tomography indicators of blood brain barrier permeability and cerebral microperfusion improve long term after carotid stenting in symptomatic patients

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Objectives: Strong interdependence between severity of microcirculation impairment and time-based perfusion parameters has been recently reported. In particular, mean transit time (MTT) appears to show good association with cerebral small vessel disease (1, 2). Blood brain barrier (BBB) damage, in turn, is considered as a main initial pathogenic mechanism in cerebral small vessel disease (3, 4).
We tested the hypothesis that absolute computed tomography (CT) markers of BBB and cerebral microcirculation would improve 36 months after internal carotid artery stenting for symptomatic carotid stenosis while results obtained 6–8 weeks after stenting procedure would yield predictive value.
Methods: We recruited consecutive eligible patients with >70% symptomatic single carotid stenosis with a complete circle of Willis and normal vertebral arteries to observational cohort study. We detected changes in cerebral blood flow (CBF), cerebral blood volume (CBV), MTT, time to peak (TTP) and permeability surface area-product (CT marker of BBB permeability, PS) before and after carotid stenting. We compared absolute differences in ipsilateral and contralateral CT perfusion markers before and after stenting. The search for regression models of "36 months after stenting" results were based on stepwise analysis with bidirectional elimination method.
Results: A total of 34 patients completed 36 months follow-up (15 females, mean age of $69.68 \pm$ SD 7.61 years). At 36 months after stenting, absolute values for CT perfusion markers had improved: CBF (ipsilateral: +7.76%, contralateral: +0.95%); CBV (ipsilateral: +5.13%, contralateral: +3.00%); MTT (ipsilateral: −12.90%; contralateral: −5.63%); TTP (ipsilateral: −2.10%, contralateral: −4.73%) and PS (ipsilateral: −35.21%, contralateral: −35.45%). MTT and PS assessed 6–8 weeks after stenting predicted the MTT (ipsilateral: $R2 = 0.867$, contralateral $R2 = 0.688$) and PS ($R2 = 0.314$ for ipsilateral side and $R2 = 0.394$) value 36 months after stenting.
Conclusions: Improvements in BBB permeability and cerebral microcirculation persist for at least 3 years after carotid artery stenting in symptomatic patients. MTT and PS measured 6–8 weeks after stenting provides predictive value with respect to MTT and PS 36 months after stenting. Diminished PS values may suggest a decline in overall oxidative and inflammatory status.

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