SCHEMA REDAZIONE PROGETTO DI RICERCA

CONCORSO PER L'ASSEGNAZIONE DI BORSE DI STUDIO DI DOTTORATO DI RICERCA

A VALERE SUI FONDI PNRR DI CUI AL D.M. 118 del 2 marzo 2023

Anno Accademico 2023/2024 Ciclo XXXIX

Dottorato di Ricerca in Biomolecular and health sciences

Tematica vincolata 'Advanced carbon-engineered organs-on-a-chip: Innovative nanotools-based platforms for brain Injury repair'

Alice Sartini

TITOLO DEL PROGETTO: 'Combining carbon-based materials and Organs-on-a-Chip for restoration of brain injury-related dysfunctions of the neurovascular unit'

RICERCA PROPOSTA

ABSTRACT

With this project we would utilize carbon-engineered Organs-on-a-Chip to culture the different elements of the neurovascular unit. The model would then be submitted to mechanical perturbation to observe the dysfunctions that are present at the level of the blood brain barrier in the case of Traumatic Brain Injury. Carbon based material have shown to increase cell functionality and connectivity and with this study we aim to observe the potential effects of these nanotools in ameliorating the neurovascular impairments in brain injury.

STATE OF THE ART

The neurovascular unit (NVU) is a complex structure composed by various elements, such as endothelial cells (ECs), neurons, astrocytes and pericytes. Each of these components is involved in maintaining the metabolic homeostasis of the central nervous system. In particular, disruptions or dysfunctions at the level of the NVU have been observed in several neurological conditions, comprising Alzheimer's disease, Parkinson disease, epilepsy, amyotrophic lateral sclerosis, strokes, as well as traumatic brain injury (TBI). The connection and interplay between the elements comprising the NVU is difficult to investigate because of its difficult reproduction in an experimental model, both in vivo and in vitro. Specifically, in vitro models have often resulted as on oversimplification of the complex dynamics present in this structure. Recently, Organs-on-aChip (OoC) have been used to replicate the NVU, as they allow for different cell types to be cultured separately while being metabolically connected, mimicking the chemical communication between the several types of cells present in the NVU. Moreover with this system it is possible to submit the cell cultures to shear stress, which mimics to the unidirectional laminar flow in the capillaries. This allows for a more realistic model, closer to the actual physiology of the NVU.

Other innovative tools in the field of neuroscience have been nanomaterials. Carbon nanotubes and graphene,

specifically, have demonstrated exceptional potential in improving cell function in vitro, making them great candidates for ameliorating neurovascular abnormalities associated with these conditions.

RESEARCH OBJECTIVES

Organs-on-a-Chip have shown great promise as models for several disorders. In particular, I am interested in observing how the use of carbon-based materials (CBM) might affect a OoC model of the NVU in the context of brain injury.

THEORETICAL AND METHODOLOGICAL FRAMEWORK

BBB disruption happens already in the hours following the TBI and it can persist for years. This includes a loss of tight junction (TJ) proteins and an increase transcytosis across the endothelial cell, which allow passage of molecules that normally wouldn't pass the barrier.

To study the involvement of each cell type in the NVU functionality and the dysfunctions caused by the TBI, we are going to develop an in vitro model of the NVU.

For this project I would use a microfluidic model which comprises the different cell types of the NVU and exposes them to blood-flow associated sheer stress. Models using cultures of BBB endothelial and glial cells are common to recapitulate the NVU in vitro but it would be interesting to add a culture of neuronal cells, which are not strictly part of the BBB but have been shown to maintain several of its properties and functions. Neurons, endothelial cells, pericytes and astrocytes could potentially be derived from human-induced pluripotent stem cells (IPSC), to recapitulate a model closer to the human condition in vivo.

The chips would be integrated with Carbon-based nanomaterials, such as carbon nanotubes, which are biocompatible and have been shown to increase neuronal cells' electrical activity, cell growth and vesicles' release.

RESEARCH DESIGN

First, we'll design a OoC with carbon based materials, to study the interactions between the different NVU components, which might be enhanced by the CBMs.

For the experiment we could realize two different OoC: a 'BBB chip', formed by endothelial cells on the bottom part and pericytes and astrocytes on the upper surface of the membrane; and a 'brain chip', containing neurons, as well as glial cells. As mentioned, these cultures would be separate while being metabolically coupled, which allows for interactions between cells.

TEER and immunofluorescent assays could be used to evaluate the BBB phenotype integrity.

Once the model is established we'll induce a mechanical perturbation and we'll observe how it affects each cell type.

Finally we'll be able to observe if CBMs are able to restore the disruptions caused by the TBI.

EXPECTED RESULTS

By incorporating CBMs to OoC we might be able to regenerate the impaired crosstalk in NVU and gain a better understanding of the mechanisms involved.

BBB dysfunction in TBI is considered a major risk factor for high mortality and morbidity and an early restoration of BBB integrity might help in reducing the number of injury-related deaths and comorbidities.

BIBLIOGRAPHY

Canfield SG, Stebbins MJ, Morales BS, Asai SW, Vatine GD, Svendsen CN, Palecek SP, Shusta EV. An

isogenic blood-brain barrier model comprising brain endothelial cells, astrocytes, and neurons derived from human induced pluripotent stem cells. J Neurochem. 2017 Mar;140(6):874-888.

Cash A, Theus MH. Mechanisms of Blood-Brain Barrier Dysfunction in Traumatic Brain Injury. Int J Mol Sci. 2020 May 8;21(9):3344.

Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park TE, Dauth S, Mannix R, Budnik N, Shores K, Cho A, Nawroth JC, Segrè D, Budnik B, Ingber DE, Parker KK. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. Nat Biotechnol. 2018 Oct;36(9):865-874.

Rago I, Rauti R, Bevilacqua M, Calaresu I, Pozzato A, Cibinel M, Dalmiglio M, Tavagnacco C, Goldoni A, Scaini D. Carbon Nanotubes, Directly Grown on Supporting Surfaces, Improve Neuronal Activity in Hippocampal Neuronal Networks. ADVANCED BIOSYSTEMS, vol. 3, p. 1-13, ISSN: 2366-7478. 2019.

Rauti R, Ess A, Roi BL, Kreinin Y, Epshtein M, Korin N, Maoz BM. Transforming a well into a chip: A modular 3D-printed microfluidic chip. APL Bioeng. 2021 Apr 28;5(2):026103.

Rochfort KD, Cummins PM. In vitro cell models of the human blood-brain barrier: Demonstrating the beneficial influence of shear stress on brain microvascular endothelial cell phenotype. BloodBrain Barrier (2019), pp. 71-98

Wang X, Xu B, Xiang M, Yang X, Liu Y, Liu X, Shen Y. Advances on fluid shear stress regulating bloodbrain barrier. Microvasc Res. 2020 Mar;128:103930.