COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

A Tetra-cell Blood-brain Barrier (bbb) Model System For High Throughput Screening Of Medical Countermeasures (mcms) For Rapid Response

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The human blood–brain barrier (BBB) is an important histological barrier that controls the interplay and molecular trafficking between the central nervous system (CNS) and the periphery. The BBB along with the neurons form a neurovascular unit (NVU). The physiological constraints of these NVUs could be investigated more closely employing the use of organoid in vitro models, with special interest on how the BBB is responding to neurological pathology and drug delivery and multiple clinical scenarios of neuropathology.
Studying organ and tissue systems in vitro has been a major hurdle for neuroscience research. Many projects that seek to further define neurological illness often have to rely on animal models to be able to replicate and observe phenomena at the organ/tissue level. This involves tedious, low-throughput rodent work. To overcome this, FDA supported the development of a “human-on-a-chip” that will allow research scientists to perform experiments on a realistic replica when testing the effectiveness of novel experimental therapies.
In this DTRA supported study, we have established an in vitro 3D human BBB model along with neurons. We have used cerebral microvascular endothelial cells, astrocytes, primary pericytes, and cortical neurons co-cultured in transwell plates. This innovative model mimics the anatomy of the human BBB and NVU, which is formed by the dynamic interaction of these four key cell types. As such, the model is expected to exhibit many of the same barrier properties: for example, the expression of intercellular tight-junction proteins including zonula occludens-1 (ZO-1), occludin, and claudin. We have performed immunocytochemistry (ICC) staining to visualize these proteins as indicators of barrier integrity. We have also established a quantitative assay to evaluate membrane disruption pre- and post-treatment with surrogate chem-bio agent. For this BBB model evaluation, we have used a peptoid-based diketopiperazine (DKP) small-molecule shuttle conjugated with acetylcholine (Ach) reactivators to quantitate permeability and therapeutic activity of conjugates. This model offers the flexibility of adding complexity to mimic the immune system as well for in vitro screening. This model can be also pre-treated with different chem-bio agents to develop exposed pathology and follow-on efficacy screening for newly synthesized MCMs and thus supports host-directed interventions to emerging threats for rapid response.