# Multi-omics analysis reveals ischemic stroke-like features in matured hiPSC-deroved neurospheroids after oxygen-glucose deprivation/reoxygenation

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Despite the high global burden of ischemic stroke on patients and society, treatment options are limited and decades of research dedicated to finding new candidate neuroprotective drugs has not led to an effective neuroprotective therapy to date. This is partially due to the lack of appropriate model systems that recapitulate human ischemic responses in vitro. To address the shortcomings of these models, we developed a 5-month-old matured, bioreactor-based, hiPSC-derived neurospheroid model to more faithfully mimic adult neural tissue and its cellular interactions. Characterization of these neurospheroids showed presence of neurons, astrocytes and spontaneous electrophysiological activity. Notably, culturing these neurospheroids in a bioreactor reduced necrotic core formation typically present in organoids cultured for prolonged periods of time. To mimic ischemic stroke-like conditions, we exposed these neurospheroids to six hours of oxygen-glucose deprivation (OGD), followed by 72 hours of reoxygenation. The release of neurofilament-l, used as a marker for neuronal cell death, significantly increased in the OGD-exposed condition compared to the control. Additionally, analysis of untargeted transcriptomics and proteomics revealed upregulation of processes related to oxidative stress after 72h of reoxygation. Moreover, alterations in developmental and inflammatory signalling as well as a distortion of cellular metabolism and neurotransmission were detected. This translates to a loss of electrophysiological network activity as demonstrated by live cell Ca2+-imaging. In addition, we demonstrated the feasibility of incorporating immune cells known to play important roles in ischemic stroke, such as microglia, macrophages and neutrophils into these neurospheroids post-OGD. With this, we created a new model system to further investigate the neuroinflammatory cascade following ischemic stroke, which can help identify new targets for neuroprotection or repair.