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BBB-on-a-chip model: new protocols and 3D observations in glass microchips

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The blood-brain barrier (BBB) structure is essential for maintaining brain homeostasis by regulating the fluid and substances transport between the bloodstream and central nervous system, while protecting against toxins and pathogens. Disruption of BBB integrity is associated with neurodegenerative diseases like Alzheimer' s and Parkinson's, in which factors such as inflammation and amyloid-β accumulation impair the barrier function. Accurate in vitro models of the BBB, in particular platforms capable capable of mimicking 3D environment, are essential for studying pathological mechanisms. The evaluation of BBB integrity involves the observation of tight junction proteins like zonula occludens-1 (ZO-1) through immunofluorescence microscopy.

In this study, we investigated the impact of three different fixation protocols, on the immunofluorescent detection of ZO-1 in the b.End3 mouse brain endothelial cell line. We also evaluated how the composition of the culture medium influences ZO-1 expression and visualization. Our results showed that Advanced DMEM cell culture medium provided a more stable environment for ZO-1 expression in comparison with standard DMEM, while methanol and acetone fixation significantly improved the signal intensity and image clarity, regardless of the culture medium used.

We further validated the protocols in a 3D microfluidic glass chip fabricated in photosensitive glass by picosecond laser assisted etching. The microchip allowed the clear visualization of tight junction architecture within a confined 3D microscale environment. Our results may contribute to either standardization of immunofluorescence workflows in 3D spaces or support the use of glass microfluidic platforms for rigorous biophysical studies of BBB integrity

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