194d A Facile and Reproducible in Vitro Blood-Brain Barrier Model

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The brain vasculature, otherwise known as the blood-brain barrier (BBB), is a formidable barrier to neurological disease treatment. This impermeable class of endothelium presents both a physical barrier through tight junctions adjoining neighboring endothelial cells and a transport barrier comprised of efflux transporters. These two assets are critical for the maintenance of brain homeostasis, but also serve to restrict drug transport from the bloodstream to the brain interstices. In fact, greater than 98% of small molecule pharmaceuticals do not cross the BBB, and no protein or gene medicines exhibit marked brain uptake without some mode of brain targeting. Therefore, a representative in vitro BBB model can be extremely helpful in preliminary screens for BBB permeable drugs and the development of noninvasive delivery strategies. Unfortunately, when grown in vitro, brain endothelial cells rapidly dedifferentiate and lose their unique impermeability and transport characteristics. This is in part due to the presence of contaminating perivascular cells that are invariably present in the brain blood vessel preparations. In addition, removal of the endothelial cells from their local inductive brain microenvironment further diminishes their in vivo character. We have validated a method that results in pure, impermeable monolayers of rat brain endothelial cells (RBEC). The translation inhibitor, puromycin, was employed to generate pure RBEC monolayers. Puromycin is efficiently effluxed from RBECs by p-glycoprotein, and thus these cells are protected while selective toxicity removes common cellular contaminants such as pericytes, smooth muscle cells, and fibroblasts. RBEC culture purities were routinely as high as 99.8% as determined by immunocytochemistry, greatly surpassing other techniques of similar complexity that were examined in parallel. The use of puromycin did not affect the localization of several tight-junction proteins (occludin and zonula occluden-1), nor did it alter trans-endothelial electrical resistance (TEER) measurements of RBEC monolayers. The barrier properties were further improved with the use of hydrocortisone, a method that had been previously shown to increase BBB characteristics in porcine and murine systems. It was found that the resistances in rat BEC cultures treated with puromycin and hydrocortisone (225±16 ohm-cm^2) were more than double those treated with hydrocortisone alone (99±19 ohm-cm^2) indicative of a decreased permeability. In addition, the RBEC morphology became more spindle-like with improved continuity of tight junction proteins at cell borders. This novel barrier model is pure, reproducible, has improved permeability characteristics, and is therefore amenable to drug permeability testing.