DEVELOPMENT OF A HUMAN *IN VITRO* BLOOD-NERVE BARRIER MODEL

EROBOGHENE E. UBOGU, M.D., NEJLA YOSEF, M.S., ROBIN H. XIA, M.D., Ph.D.

NEUROMUSCULAR IMMUNOPATHOLOGY RESEARCH LABORATORY, DEPARTMENT OF NEUROLOGY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TEXAS, UNITED STATES OF AMERICA.

ABSTRACT

Phenotypic and functional differences exist between vascular endothelium from different tissues and between microvascular and macrovascular endothelial cells from the same tissue. Little is known about cellular interactions at the blood-nerve barrier (BNB).

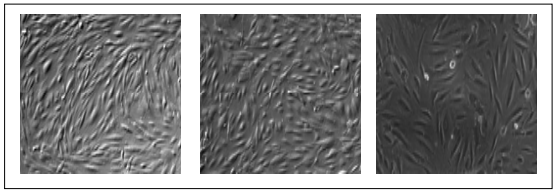
An *in vitro* BNB (IVBNB) model was developed using primary human endoneurial endothelial cells (pHEndECs) freshly isolated and purified from decedent sciatic nerves via endoneurial stripping, connective tissue enzymatic digestion and density centrifugation.

pHEndECs are spindle shaped, contact inhibited, differentiate to form capillary-like tubes, are Ulex Europaeus Agglutinin 1 and von Willebrand Factor positive and endocytose acetylated low density lipoprotein. They also express alkaline phosphatase, γ-glutamyl transpeptidase, glucose transporter-1, p-glycoprotein and low levels of cellular adhesion molecules. Culturing pHEndECs on collagen coated transwell inserts was used to develop the IVBNB. High transendothelial electrical resistances (~160 Ω.cm2; maximal 12 days after seeding) and low solute permeability to fluoresceinated high molecular weight (70 kDa) dextran (~0.7%) were seen. Electron microscopy demonstrated intercellular tight junctions and 50-100 nm-diameter pinocytic vesicles.

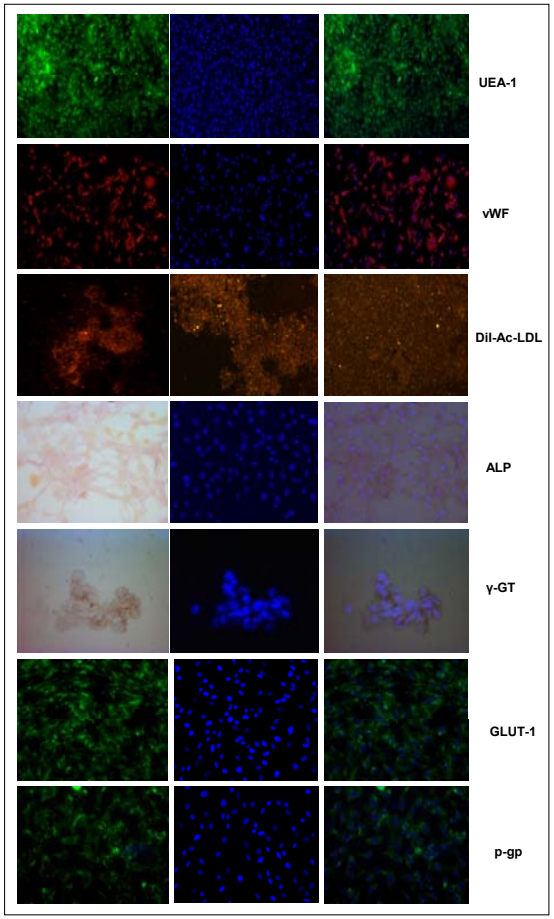
These features are consistent with the BNB. This model provides an avenue to study mechanisms of leukocyte entry into peripheral nerves.

Human Sciatic Nerve and Endoneurial bundles

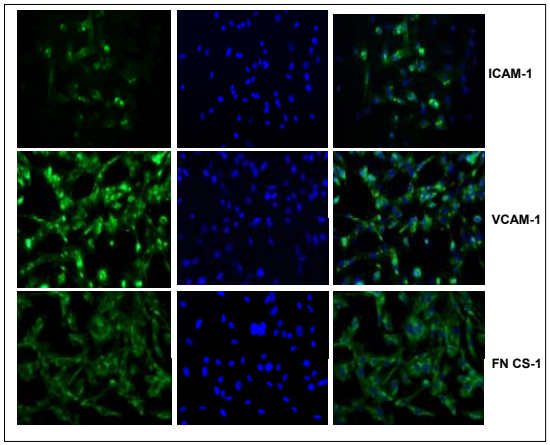
Primary Human Endoneurial Endothelial Cells (pHEndECs)



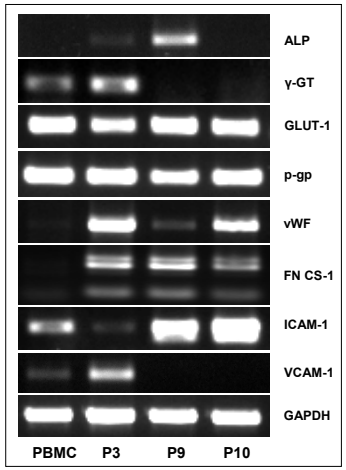
Phenotypic Characterization of pHEndECs



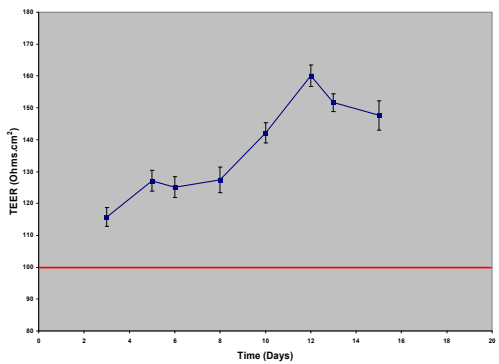
Cellular Adhesion Molecules

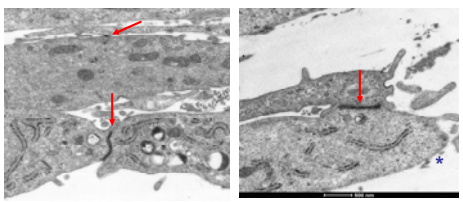


Polymerase Chain Reaction

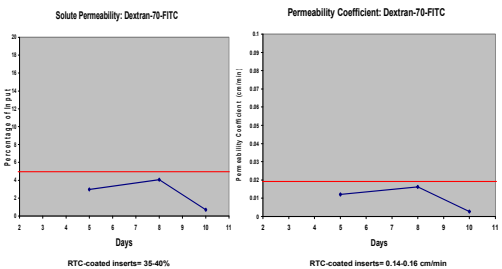


Primary HEndEC Transendothelial Electrical Resistance





TRANSMISSION ELECTRON MICROSCOPY SHOWING INTERCELLULAR TIGHT JUNCTIONS AND PINOCYTIC VESICLES *IN VITRO*.



POTENTIAL APPLICATIONS OF THE IVBNB

- Endogenous solute/ macromolecular transport studies
- Drug transport studies
- Toxin/ xenobiotic influx/ efflux studies
- Mechanisms of leukocyte transmigration
- Microbial invasion processes
- Chemokine translocation/ presentation mechanisms

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