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Identifying molecular techniques amenable to a porcine 3D retinal study model to examine structural degeneration and early molecular deregulation relevant for diabetic retinopathy

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Diabetes is a chronic metabolic disease associated with micro-vascular complications including diabetic retinopathy (DR). Chronic high glucose concentration is known to increase the incidence and severity of DR, however, the sequence of molecular events is complex and the precise mechanisms involved are still debated. This study presents a 3D porcine retinal model designed to examine the early molecular deregulation of retinal tissue. Porcine retinal tissue was extracted from individual ocular globes (n≥6) donated by a local abattoir within 2 hours post-mortem. Retina were cultured in DMEM within an agarose (0.5%) + collagen (0.5 mg/ml) co-gel and maintained in a humidified atmosphere with 95% air and 5% CO2 at 37°C. Retinal tissue fixed in 10% neutral buffered formalin (NBF) and processed for paraffin embedding and sectioning. Tissue sections (10 µm) were stained with haematoxylin and eosin (H&E) and immunohistochemistry (IHC) was carried out with anti-HIF1α (1:800), anti-GFAP (1:100), and anti-CD31 (1:100). In addition, retinal tissues were harvested at different time points 0/4/24 hours and RNA was extracted using TRIzol reagent to identify the feasibility of using the model for these of gene expression. Use of H&E enabled visualization of the retinal structure and IHC demonstrated the expression of specific proteins. In contrast, RNA electrophoresis indicated that there was partial degradation of the RNA which would exclude this approach from meaningful further analysis. Further refinement of the model may be possible to improve RNA quality. Thus the 3D retinal model has the potential for studying early deregulations in DR.

Biography

Benedicta U Iwuagwu is currently a second year PhD student in the School of Pharmacy & Life Sciences and obtained her Post-graduate degree in Research Methods at Robert Gordon University.

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