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Changes in the Transcriptome of Glucose-Challenged Human Glomerular Epithelial Cells Following Treatment with Cannabinoid Derivatives.

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Project Rationale & Goals

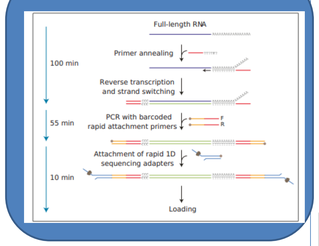
- Kidney disease affect 35.5 millions of Americans each year. Podocytes are cells fund on the glomerulus of the kidney. These cells also referred to as human glomerular epithelial cells (HGEC) in the kidneys that are responsible for filtration of substances in the blood and urine. High level of glucose can cause these cells to malfunction and thus increasing the risk of diseases such as diabetes.
- Cannabinoids, the active compounds found in the cannabis plant, have gained attention for their potential medicinal benefits. The two most well-known cannabinoids are tetrahydrocannabinol (THC) and cannabidiol (CBD). Some of the medicinal benefits associated with cannabinoids include: pain relief, anti-inflammatory effects, epilepsy and seizures, neuroprotective properties against neurodegenerative diseases such as Alzheimer's, Parkinson's, and multiple sclerosis, skin conditions such as psoriasis, eczema, and acne.
- More research is needed to fully understand the efficacy and safety of cannabinoids. Current research on medicinal cannabinoids is revealing promising results across various health conditions, while also highlighting gaps and the need for further study. Key areas of research include pain management, anxiety reduction, and oncology supportive care.
- The healings effects of cannabinoids, specifically, cannabidiol, on abnormal human glomerular epithelial cells as well as the differentially expressed genes between abnormal (post diabetic) HGEC and normal (prediabetic)

Materials & Methods

Culturing of Pre and Post Diabetic HGEC Cells and treatment of CBC,CBD,CBG

To compare and contrast the changes in morphology, physiology of cells in onset diabetic	
(HGEC:5 mM-to-25 mM/2w)	Cells cultured in 5mM[ ] of glucose for 3 weeks then switched to 25mM glucose [ ] for 2 weeks
(HGEC:5 mM-to-25 mM/6w	Cells cultured in 5mM[ ] of glucose for 3 weeks then switched to 25mM glucose [ ] for 6 weeks
HGEC:5 mM-to-25 mM/18w)	Cells cultured in 5mM[ ] of glucose for 3 weeks then switched to 25mM glucose [ ] for 18 weeks
(HGEC:25 mM-to-5 mM/18w)	Cells cultured in 25mM[ ] of glucose for 3 weeks then switched to 5mM glucose [ ] for 18 weeks
(HGEC:25 mM/6w-to-5 mM/4w)	Cells cultured in 25mM[ ] of glucose for 6 weeks then switched to 5mM glucose [ ] for 4 weeks
HGEC:25 mM/18w-to-5 mM/4w)	Cells cultured in 5mM[ ] of glucose for 18 weeks then switched to 5mM glucose [ ] for 4 weeks
NHGEC:5mM/3wk- control, normal HGEC cells	Cells cultured in 5mM[ ] of glucose for 3 weeks

RNA Extraction



Cell Viability (MTT Assay)

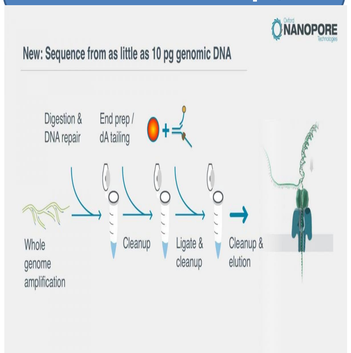
ONT Nanopore sequencing (for quantifying nucleotide bases in transcript)

Assay protocol

An overview of the steps is shown in the following table:

Step	Description	Volume/well [µl]	Time [Hours]	Temperature [°C]
1	Perform tissue culture using 96-well, tissue-culture grade, flat-bottom microplates.	100	24 – 96	+37
2	Add MTT labeling reagent and incubate in a humidified atmosphere.	10	4	+37
3	Add Solubilization buffer and incubate in a humidified atmosphere.	100	Overnight	+37
4	Evaluate microplate using an ELISA reader at 550 to 600 nm with a reference wavelength of >650 nm.			

Spectrophotometer to measure wavelengths



Results & Discussion

Figure 1 showing the cell viability after different concentrations [uM] of CBG treatment of the different human epithelial glomerular cells (HGEC). Numerical values in legend depicts the IC50,highest dose that all cells do not die.

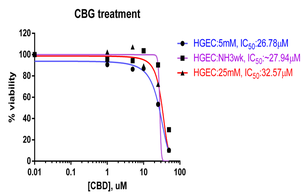


Figure 2 showing the cell viability after different concentrations [uM] of CBC treatment of the different human epithelial glomerular cells (HGEC). Numerical values in legend depicts the IC50,highest dose that all cells do not die

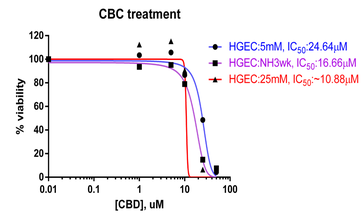


Figure 3 showing the cell viability after different concentrations [uM] of CBD treatment of the different human epithelial glomerular cells (HGEC). Numerical values in legend depicts the IC50,highest dose that all cells do not die

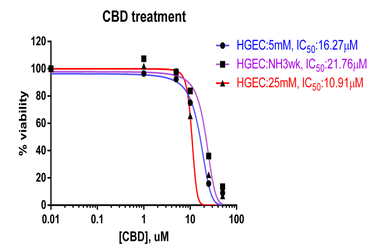


Figure 4 showing the cell viability of normal glucose level [5uM] prediabetic HGEC where the presence of mitochondrial enzymes produce a darker color showing living cells and lighter(yellow color) less enzymes in dead cells. This is after the treatment of the cannabinols CBD, CBC and CBG.

Figure 5 showing the cell viability of normal HGEC, no glucose, where the presence of mitochondrial enzymes produce a darker color showing living cells and lighter(yellow color) less enzymes in dead cells. This is after the treatment of the cannabinols CBD, CBC and CBG

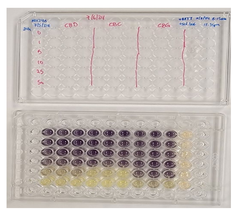


Figure 6 showing the cell viability of high glucose level [25uM]post diabetic HGEC where the presence of mitochondrial enzymes produce a darker color showing living cells and lighter(yellow color) less enzymes in dead cells. This is after the treatment of the cannabinols CBD, CBC and CBG.

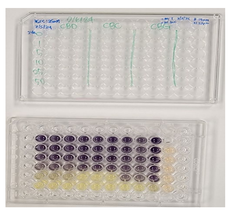
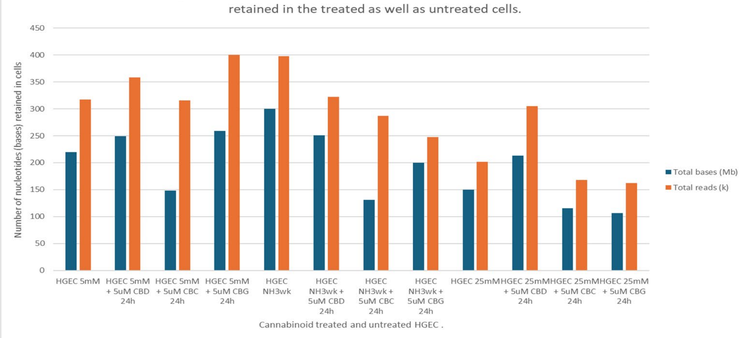


Chart showing the results of ONT sequence to depict the number of RNA nucleotides(bases) retained in the treated as well as untreated cells.



This research gives attention to the physiology of kidney cells such as the human glomerular epithelial cells and podocytes located in the glomerulus of the nephrons. Nephrons are the structural components of the kidneys responsible for the filtration of blood and the production of urine. Glomerular (Bowman's) capsules or glomerulus contain flattened, thin epithelial cells functional for filtration. Podocytes are cells in the Bowman's capsule in the kidney that wrap around the capillaries present in the glomerular capsule. The podocytes are also part of the layer of epithelial cells in the Bowmans capsule.

Podocytes are differentiated cells and modify and help form slits in the in the glomerular cells that allows filtration of substances from the blood to take place.

Glucose levels can also affect the function of kidney epithelial cells where high glucose puts a cell in a diabetic state. In this paper, normal blood glucose levels for prediabetic human glomerular epithelial cells to exist were mimicked at 5mM concentrations and post diabetic HGEC was mimicked at 25mM. Cells were alternate between 5mM and 25mM for different periods of time in order to observe how the function as well as morphology will change and if cells can go from prediabetic to post diabetic and if the reverse can happen. The kidney epithelial cells were incubated in vivo in cell media and the different concentrations of glucose were alternated for different amounts of time and biomarkers were measured to show modulation of the cells. The effect of cannibinols, CBD,CBG and CBC, on onset diabetic HGEC cells were studied. This is upcoming and ongoing research.

Next Steps

- The differential analysis of the RNA and cDNA of the samples to be able toe to identify and quantify full-length transcripts and observe differentiation. We did not get enough time to complete this.

References

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