EFFECT OF HYPERGLYCEMIA ON EPCS FUNCTION AND REGENERATIVE ABILITY

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Introduction

Diabetes is characterized by elevated glucose levels in the blood or "hyperglycemic conditions" due to the defect in insulin action or secretion. These conditions increase the risk of cardiovascular complications as they impact vascular endothelial cells causing vascular dysfunction. Vascular endothelium repair occurs by resident endothelial cells, however these cells have limited proliferation and self-repair ability. It has been suggested that endothelial progenitor cells (EPCs) participate in the repair and maintenance of vascular endothelial cells as well as assisting in the generation of blood vessels. EPCs have been classified into two different subtypes according to their emergence in culture; early EPCs and late EPCs (or Blood outgrowth endothelial cells, BOECs); and these types differ in their origin, phenotype and differentiation abilities. While early EPCs are broadly studied in diabetes, the role of late EPCs/BOECs and the mechanisms of their repair are not clear. Late EPCs/BOECs are known to have potential regenerative abilities and might constitute a potential cure to protect form cardiovascular complications in diabetic patients. This study aims to view the side effects of hyperglycemic environment on BOECs viability, cytotoxicity, and regenerative functions.

Hypothesis and Aims

Hypothesis: Hyperglycemia affects EPCs/BOECs function and impairs their regenerative and homing potential.

Aims:

Test the effects of hyperglycemia on BOECs viability using Alamar blue.
Study the effect of hyperglycemia on BOECs response to shear stress
Test BOEC's repair abilities following chronic exposure to hyperglycemic environments'
Study the effects of chronic hyperglycemic conditions on endothelial cell barrier function

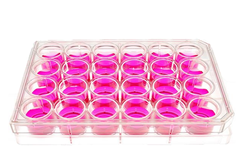
Methods

Proliferation assay

BOECs were seeded into 96 wells plates, and treated with normal glucose media (NG, 5mM) or HG (25mM) for 72hr, followed by alamar blue viability assay.

Shear stress

BOECs cultured in NG and HG media were exposed to shear stress using an orbital shaker, and cells were imaged.



Angiogenesis assay

Treated BOECs were cultured on Matrigel, and tube formation was estimated following 16hr of culture.

Cell migration and Barrier function assays

Treated BOECs were seeded into transwells and cell migration was estimated following 16hr of culture. For barrier function, the migration of fluorescently-tagged dextrans was tracked.

Results

- Exposing BOECs to HG media for 3 days resulted in a significant reduction in viability (Fig 1)

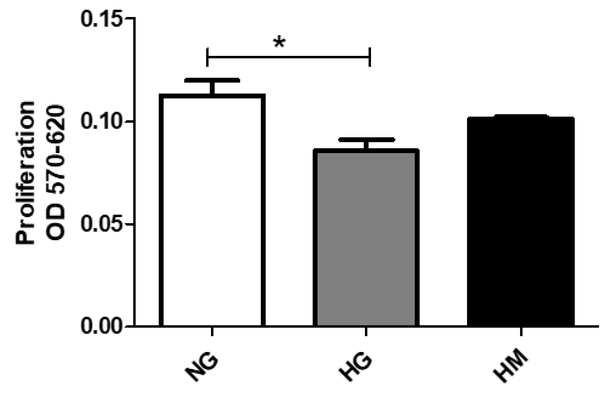


Figure 1. *Cell viability assay measured as the reduction in alarm blue stain. Exposure of BOECs to HG reduced viability, while using mannitol control didn't affect proliferation. n=3 using one BOEC isolate. Analysis was done using one way ANOVA.*P<0.05.*

- Cell alignment and elongation under hyperglycemic conditions in response to shear stress

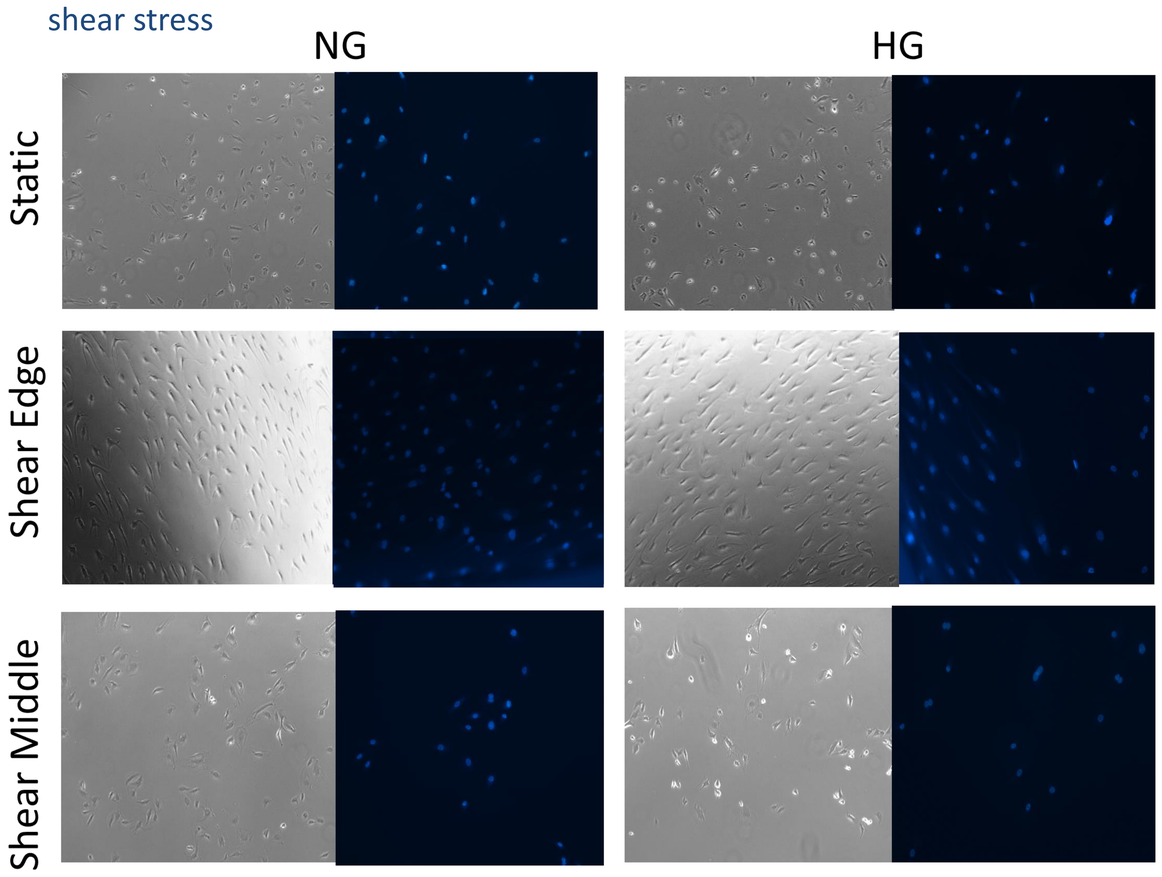


Figure 2. *Cell alignment and morphology under shear stress for NG vs HG under light microscope and using DAPI stain. The cells on the edges were under unidirectional stress in the orbital shaker while the center was left disturbed and the figure clearly shows the difference in cells morphology under both conditions.*

- Exposing BOECs to HG media for 3 days didn't affect tube formation. However, the mean mish size was affected in HG treated cells.

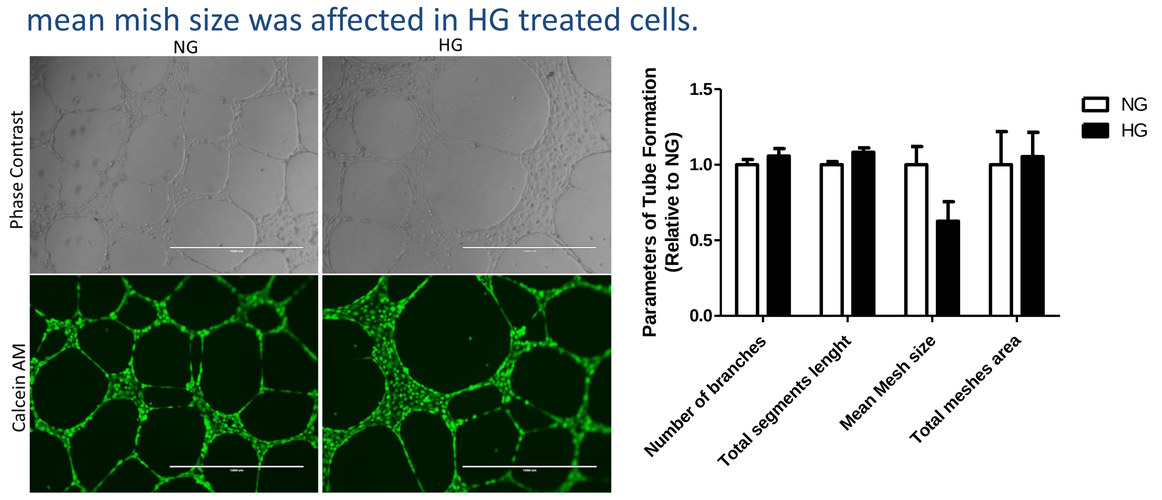


Figure 3. *Tube formation assay measured in Matrigel. BOECs treatment with HG media for 3 days didn't affect tube formation. Tube formation parameters were corrected to NG. n=3 using one BOEC isolate. Analysis was done using one way ANOVA.*P<0.05.*

- Exposing BOECs to HG media for 3 and 6 days significantly reduced BOECs migration ability through transwells.

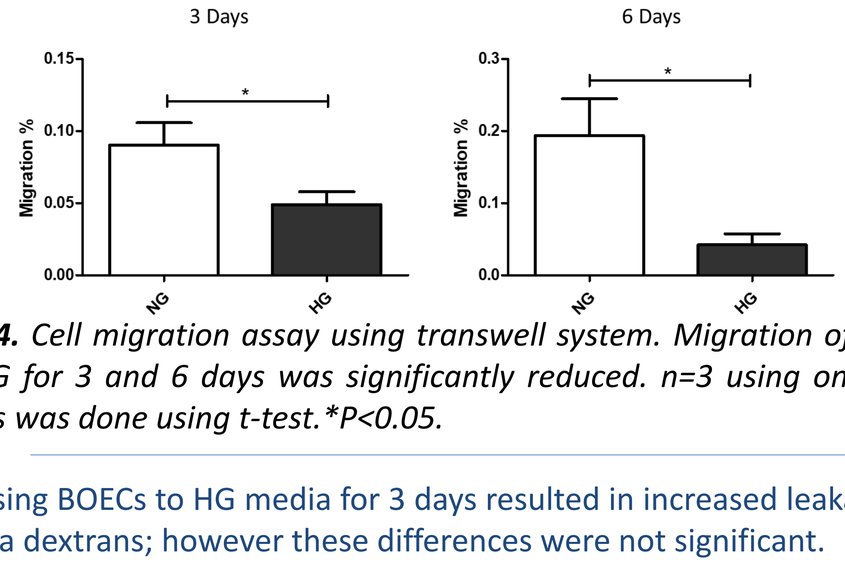


Figure 4. *Cell migration assay using transwell system. Migration of BOECs treated with HG for 3 and 6 days was significantly reduced. n=3 using one BOEC isolate. Analysis was done using t-test.*P<0.05.*

- Exposing BOECs to HG media for 3 days resulted in increased leakage of 150 and 20KDa dextrans; however these differences were not significant.

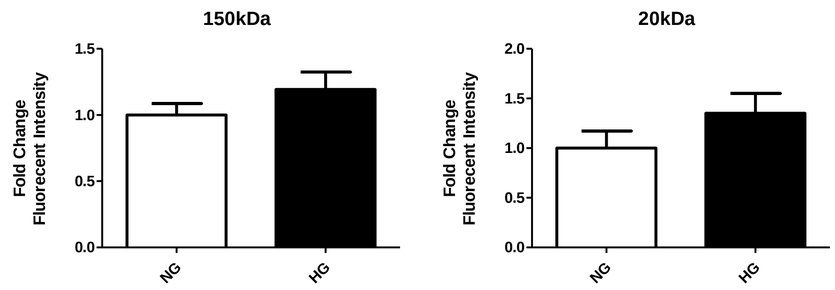


Figure 5. *Barrier function as measured by the leakage of dextrans through cell monolayers cultured on transwell systems. n=3 using one BOEC isolate. Analysis was done using t-test.*

Conclusion

In conclusion, hyperglycemia causes a plethora of negative effects on the endothelial cells, it decreases cell migration and barrier function. These functions are vital and are strongly depended on for the regeneration of the blood vessels as well as the control of the exchange of nutrients and waste between blood and tissues, and protection form pathogens. These effects are consistent with some symptoms shown by diabetic patients with cardiovascular complications. The future prospects for this area of research is to investigate the pathways of cell signaling involved in the vasculogenesis and angiogenesis in order to find ways to reverse or reduce the effects of the hyperglycemia on these cells by the use of biological molecules or drugs for treatment, especially in the subject of the impaired reparation and regeneration processes of these endothelial cells that are vital for the strength and integrity of the blood vessels.

References:

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