Modeling Diabetic Endothelial Dysfunction *in vitro*

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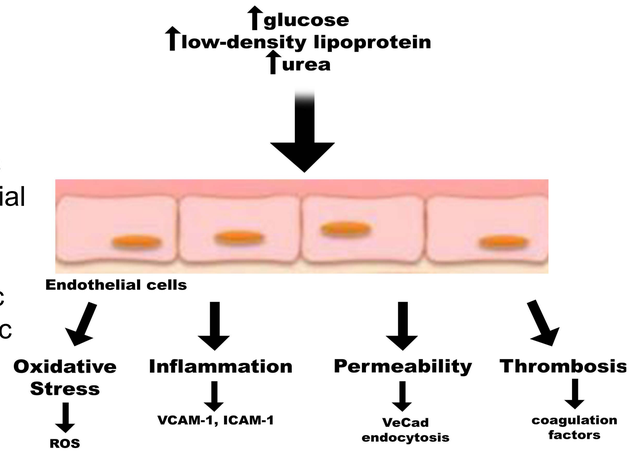
Introduction

Diabetes is one of the major risk factors for cardiovascular diseases, which is mediated with vascular endothelial dysfunction.

Hypothesis:
We hypothesize that the introduction of environmental factors, such as elevated glucose and urea, to a healthy patient's endothelial cells will induce a phenotypic change like that of a diabetic patient's endothelial cells.

Project Aims:
Aim 1: Establish *in vitro* disease models for diabetic endothelial cell dysfunction.
Aim 2: Develop precision therapeutic strategies for diabetic endothelial cell dysfunction.

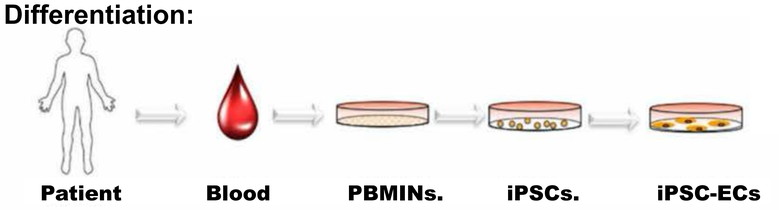


Materials and Methods

Blood Collection:

- 62-year old Caucasian female, AMH04
  - No diagnosed history of cardiovascular disease.
- 56-year old Black male, PAD08
  - Diagnosed with diabetes, peripheral arterial disease, and underlying coronary artery disease.

Induced Pluripotent Stem Cell-to-Endothelial Cell (iPSC-EC) Differentiation:



- Peripheral blood was reprogrammed with Sendai viruses encoding the Yamanaka factors and differentiated following the path shown above.

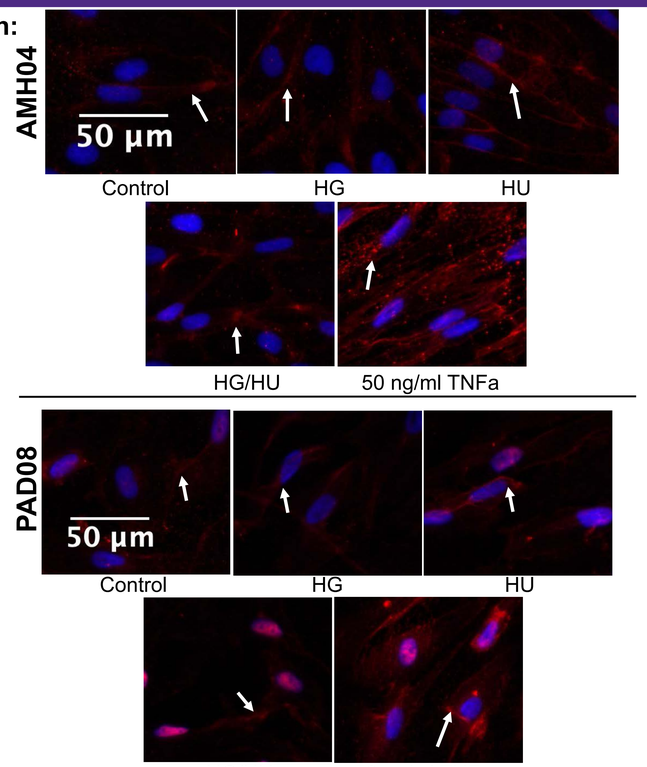
Treatment Conditions & Evaluations:

Treatment	[Glucose]	[Urea]	Time
Control	5 mM	0 mM	5 days
High Glucose (HG)	25 mM	0 mM	5 days
High Urea (HU)	5 mM	20 mM	2 days
HG/HU	25 mM	20 mM	2 days

Evaluation	Technique
Oxidative Stress	ROS generation assay
Inflammation	Immunofluorescence
Permeability	Immunofluorescence

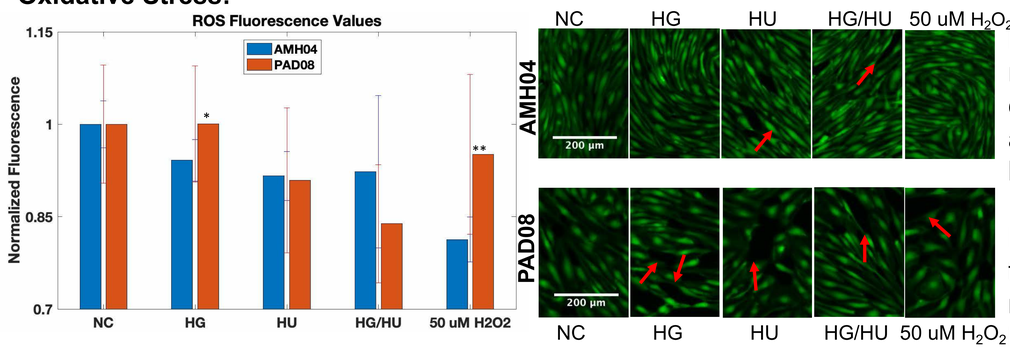
Results

Inflammation:



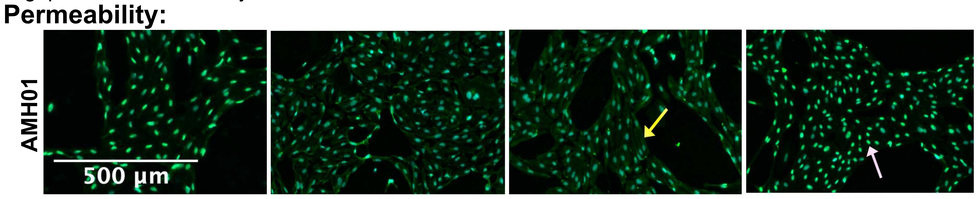
Immunofluorescence for AMH04 and PAD08 p3 iPSC-ECs with VCAM-1 (red) and nuclei (blue). Inflammatory cytokine, TNFa, was used as a positive control. White arrow denotes examples of VCAM-1 localization in the membrane.

Oxidative Stress:



Reactive oxygen species (ROS) generation assay on AMH04 and PAD08 p3 iPSC-ECs (Left). Fluorescence values are normalized to each cell line's negative control. 50 uM H_2O_2 was used as a positive control. Statistical significance is indicated by * for a p-value <0.05 or ** for a p-value <0.01. Green fluorescence (right) represents localization of ROS within the cytosol. Red arrows denote gaps within the monolayer.

Permeability:



Immunofluorescence of AMH01 p2 iPSC-ECs with VeCad (green) and nuclei (cyan). Yellow arrow denotes VeCad localization in the cytosol. Pink arrow denotes VeCad localization in the nucleus.

Discussion

Treatment groups: Concentrations for glucose (25 mM) and urea (20 mM) were based off *in situ* plasma levels for type II diabetic (TIID) patients. For inflammation, TIID patients have higher levels of circulating TNFa, an inflammatory cytokine. For oxidative stress, 50 uM of hydrogen peroxide was used as a positive control.

Evaluations:

Inflammation: As expected, we see consistent increases in expression of VCAM-1, a surface adhesion molecule, in the positive control (50 ng/ml TNFa) treated groups. This can be attributed to inflammatory processes happening within the cell line. Qualitatively, the immunofluorescence for VCAM-1 shows, as expected, stronger membrane expression of VCAM-1 in the positive control groups for both cell lines. We also see a stronger signal of VCAM-1 in the HU group, suggesting we should continue to incorporate this treatment in our studies.

Oxidative Stress: In the positive control for this study, we see decreased generation of ROS. This decrease may be representative of cell detachment. Ensuring a complete monolayer for this assay is essential.

Permeability: Qualitatively, we see the most endocytosis of vascular endothelial cadherin (VeCad) in the HG/HU group. This process is representative of decreased barrier function and increased endothelial cell permeability.

Limitations: The main limitations in this study are the N number of biological replicates and the number of patient-derived cell lines. This study presents a proof-of-concept, after which, we will expand to other patient-derived cell lines.

Future Work: We seek to translate this model to a high-throughput system. This new system will simultaneously run several pharmacological agents on a patient's iPSC-ECs sample. We see the application of this model improving the quality of care of diabetic patients through facilitation of pharmacological treatment selection.

Conclusion:
Overall, the qualitative effect of the combination (HG/HU) treatment group resulted in the most apparent effect on permeability, while the HU group appeared to strengthen membrane expression for proteins involved in inflammation.

References & Acknowledgements

This work is supported by the American Heart Association (AHA).
Image in Introduction from *Lonza Bioscience.*