I. Abstract
Age related macular degeneration (AMD) is "the leading cause of severe vision loss in adults over age 50" and results from damage to the retina. Wet AMD occurs when vascular cells beneath the retina being to grow, disrupting the nutrient supply chain to the photoreceptors, essentially starving and killing them. The exact mechanisms behind this vascular growth is unknown, as is the role that aging plays in the development of this disease. In this study, three forms of cellular stress were studied: ultraviolet radiation, oxidative stress, and mechanical stress. The purpose of this study was two-fold. One, to see if development of simple methods of exposure to the above stimuli would result in changes in cellular function. Two, to determine if various markers of RPE cell damage and angiogenesis would be produced after cellular stress.

II. Introduction
- In Wet AMD, blood vessels disrupt the retinal pigment epithelium (RPE), a monolayer of cells that support the photoreceptors, leading to vision loss.
- A key protein associated with the cause of Wet AMD is VEGF (vascular endothelial growth factor), which is involved in the formation of vascular cells.
- Previous research, conducted by Farhad Farjood of the Biological Engineering department, indicated that the loss of tight junctions between RPE cells is associated with an increase in the production of VEGF.
- Thus, in this study, the key markers of cellular stress to be tested for were VEGF production and damage to tight junctions.

III. Methods
Trials conducted with ARPE-19 cells after 1 week of growth. All cells incubated for 24 hours after exposure.

Oxidative Stress
- 4.5 microliters of 3% Hydrogen Peroxide was added to four 3ml wells of a six well plate with ARPR-19 cells, with control cells in the two remaining wells.

Mechanical Stress
- The tip of a glass pipette was used to stretch the membrane of trans wells with ARPE-19 cells. Control cells grown were on unstretched membranes.

Ultra Violet Radiation
- ARPE-19 cells were exposed to UV light for 2 hours in a fume hood. Control cells received no UV light exposure.

IV. Results
Figure 1. TEER Tests
Trans Epithelial Electrical Resistance (TEER) was recorded for both the UV and Oxidative stress trials (Figure 1). Higher TEER values indicate better tight junction formation. Mechanical stress results are yet to be obtained.

Figure 2. VEGF ELISA Assay
Relative amounts of VEGF in the media was determined via an ELISA assay. In Figure 2, higher absorbance values correspond to greater amounts of VEGF.

V. Conclusions and Future Research
- Only the UV trials resulted in elevated levels of VEGF, but only the oxidative and mechanical stress trials resulted in loss of tight junction formation.
- It is possible that VEGF levels were low for the oxidative and mechanical stress cells because they were damaged so much that many cellular functions were lost, including VEGF production.
- In future research, oxidative and mechanical stress trials should be repeated but with lower concentrations of hydrogen peroxide and less severe stretching of the membrane. TEER tests should also be repeated with older colonies for higher values.

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Figure 3. ZO-1 stained green and cell nuclei stained blue. (A,B) Control, (C,D) Oxidative stress, (E,F) Mechanical stress, (G,H) UV exposure.

Confocal Imaging of Cellular Junctions